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CONTENTS

VOLUME 4 *March* 1954 NUMBERS 1, 2

FERTILIZATION IN *PHLEBODIUM AUREUM* J. SM.

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Introduction

This investigation was undertaken to explore certain morphogenetic trends in the development of the fertilized egg of *Phlebodium aureum* J. Sm. (listed by Copeland, 1947, as identical with *Polypodium aureum* L.). In the course of developing techniques of culture to supply needed quantities of adequately mature prothallia for the experimental studies, certain gaps became obvious in the information in applying the classical accounts of the life-history of ferns to this species.

The filling of these gaps, particularly as to adding details and determining the timing of events, was necessary in the controlled fertilization required in the morphogenetic study. It is believed that these findings may be of interest to persons using ferns for research or for instructional purposes.

This paper deals with the elaboration of the life-cycle of *Phlebodium aureum* (through the first division of the embryo) in the course of which these new facts are fitted into their proper places. The experimental studies on the embryo proper are reported elsewhere.

Materials and Methods

Phlebodium aureum J. Sm. is a common greenhouse and horticultural fern that is native to most of tropical America. The plant was chosen for this study as it was conveniently available and produced spores throughout the year in the greenhouse, and also because it is a member of the Polypodiaceae, one of the more highly evolved families of ferns.

Spores were collected as needed from mature plants in the greenhouse at the Harvard Biological Laboratories. Single large fronds with mature sporangia were selected after examination with a hand lens, washed in a spray of tap water to remove foreign spores, and left to dry at room temperature between sheets of clean paper. Quantities of the yellow, ripe spores were discharged from the sporangia within three days. These were used immediately or stored in the refrigerator at temperatures above freezing. Spores of this species were found to germinate best when fresh.

Spores were satisfactorily germinated and prothallia in quantity grown on both soil and nutrient agar media (hereafter referred to as soil cultures and agar cultures). Successful cultures were grown and maintained throughout the year.

For the soil cultures, the common practices of the greenhouse grower were followed. Sifted coarser soil was placed in clay pots and covered with a shallow layer of fine rich soil. The soil-filled pots were autoclaved for two hours and allowed to cool thoroughly under cover. Dry, unsterilized spores were dusted very lightly over the moist soil. Glass bell jars were applied as covers, and all subsequent watering was done from the bottom. The cultures were kept in the greenhouse at a minimal temperature of 68°F. and under a slat screen constructed to admit about 30 per cent of the direct light. The light intensity ranged up to a maximum of 200 foot-candles at mid-day.

Visible germination occurred on the soil within two weeks, and prothallia grown in thin, uncrowded cultures were sexually mature in about two months. Young embryos could then be produced in quantity, and sporeling plants were themselves mature and bearing sporangia a year after the first spores were planted.

Occasionally the cultures were attacked by fungal and algal contaminants. Flooding the cultures for a minute or so with a solution of potassium permanganate (200 mg. per liter of water) was generally enough to keep the contaminants in check. The treatment was less effective for controlling moss protonema. (Nebel, 1946, used a " medium pink " solution of potassium permanganate for similar protection of gametophyte cultures.)

Soil-grown prothallia were generally more vigorous than those grown on nutrient agar media. They were used extensively in the investigations.

The nutrient agar medium used was that described by Knudson (1940). This medium, with its two per cent sucrose, was quite satisfactory for production of vigorous prothallia. In general, the agar culture technique employed was that described by Hires (1940) and Nebel (1946). Freshly autoclaved medium (200 cc. in 500 cc. Erlenmeyer flasks) was cooled to form sloping surfaces. The spores were sterilized in a small test tube containing a solution of freshly mixed and filtered calcium hypochlorite as described by Wilson (1915). The tube was shaken vigorously during the process. Sterilization was essential for successful cultures on the agar medium, as the early and rapid growth of fungi in contaminated flasks immediately destroyed the young and slower growing prothallia. Twenty to thirty minutes in the solution was found adequate for sterilization. Although no statistical study was made, it was evident that little or no reduction in viability of the spores resulted from such treatment. Knudson (1940) found no essential decrease in the viability of spores of certain species of ferns in periods of sterilization up to 50 minutes.

The spores were removed directly from the surface of the sterilizing liquid (or collected on sterile filter paper) and spread as thinly as possible over the agar surface. A flamed wire loop was used for some of the transfers, although more uniform distribution of spores was possible through use of a small autoclaved camel's hair brush. All plantings were made in a thoroughly steamed transfer room.

Relatively few spores were required for a series of cultures. An excess was easily obtained, and subsequent maturity in the crowded condition was sharply affected. As known from workers as early as Hofmeister (1851), dense cultures tend toward continuation of the filamentous state and the maintenance of the antheridial sexual condition. Dense growths on the agar were used throughout these investigations to provide antheridial prothallia for fertilization whenever desired.

The agar cultures were kept in indirect light at room temperature. Supplemental light from ordinary bulbs for two or three hours daily increased growth significantly in the winter season. Sterile water can be added as needed to replace that lost by evaporation through the cotton plug. The use of thin sheets of polyethylene plastic (Plaxpak, 0·001″) over the mouth of the flask will greatly lessen the water loss.

Germination occurred in the agar cultures in about six days (as determined with the microscope). Full maturity of both antheridia and archegonia was reached in uncrowded agar cultures within three months with either long days or through use of supplemental lighting. (A light intensity of about 50 foot-candles at the surface of the flask was used.)

Observed fertilization was carried out at room temperature by placing mature archegonium-bearing prothallia in a few drops of tap water in the cavity of a hollow-ground glass slide. Antheridia-bearing prothallia were placed in the water near by, or the sperms were transferred as desired from other drops of water with a medicine dropper. Ordinarily, the process was observed through the binocular microscope at a magnification of 125×.

Antheridial and archegonial dehiscence and the dynamic process of fertilization itself were photographed on 16 mm. motion picture film with the Bausch and Lomb photomicrographic apparatus. The filming was done at the normal speed of 16 exposures per second, using a 10× or 20× objective as desired with a 3× ocular. The object being photographed was seen directly through the viewer at a total magnification of 150×. The pro-

thallium was held in place in the water by a piano wire clamp, attached to a wood block, which was cemented to one end of the slide. Detailed study of the projected films and the inspection of individual frames contributed measurably to the extent and accuracy of the study of many aspects of the fertilization process.

The prothallia were examined for embryos under a dissecting microscope, specially mounted on a table provided with a foot-operated focusing control. Magnifications commonly used were 75× to 115×. Both top and bottom lighting for the object was used. The focusing control was indispensable in the later dissections of the embryo and the attendant morphological study which necessitated the free use of both hands. The lighting for the dissections was significantly enhanced by the use of the 40 watt "Zirconarc" photomicrographic light; made by the Fish-Schurman Company. This point-source of light proved highly effective in observation of minute characters, in making delicate incisions, and in preparing photomicrographs from fixed material.

Prothallia and young sporophytes desired for sectioning were fixed in the chromic acid-acetic acid preparation as recommended by Johansen (1940). The material was dehydrated in the ethyl alcohol-normal butyl alcohol series of Pratt and Wetmore (1951), and embedded in 56°-58° rubberized paraffin (Fisher Tissuemat). The sections were commonly cut at a thickness of seven microns and stained by the iron alum-hematoxylin method outlined by Wetmore (1932).

Observations and Results

Since the work of Hofmeister (1851), the sequence of events in the life-history of the fern has been well established. Succeeding workers have added their contributions to this growing and popular body of botanical knowledge, one that has long since become classical even for elementary courses in botany. Among these investigators are Hanstein (1865), Strasburger (1869), Kny (1872), Bauke (1876), Goebel (1887), Campbell (1887, 1892), Shaw (1898), and many others.

In substance, the life-history as studied in this species is as follows:

1. Mature fronds of *Phlebodium aureum* bear on their undersides special spore-bearing structures, the sporangia, which occur in groups known as sori. From these sporangia are shed the yellow, kidney-shaped spores, through the functioning of a dehiscing mechanism, the annulus.

2. These spores, on a moist and moderately warm substrate, germinate and initiate development of the prothallium. Under favorable conditions the young prothallium, at first more or less filamentous, becomes somewhat heart-shaped in a few weeks when mature and develops sex organs, the antheridia and archegonia.

3. Characteristically in *Phlebodium aureum* the antheridia precede the archegonia in development, and are located generally among the rhizoids of the prothallium. Archegonia then appear more apically on the thickened portion of the prothallium, the cushion, so that the plant is now for some time morphologically monoecious. In the absence of fertilization of one or more of the eggs, each borne in an archegonium, the continued development of the prothallium is toward the production of only the female sex organs. This development is directly subject to variations induced by a varied environment. The appearance and maturity of the archegonia after that of the antheridia favor cross-fertilization, although normally there is a period when self-fertilization is possible.

4. Mature antheridia and archegonia open in the presence of water which may cover the lower surface of the prothallium and serve as a veritable culture medium for the process of fertilization. With the bursting of the archegonia and consequent extrusion of material from the neck canal, the now free-swimming sperms become chemotactically oriented toward the mouth of the archegonium and the opened canal. A number of sperms may then enter the canal and proceed to the venter. Here in a few minutes one sperm enters the egg cell, eventually to effect fertilization.

5. After fusion of the male and female nuclei within the egg, the resultant embryo tends to divide parallel to the axis of the archegonium. Preceding and attending this activity of the young embryo, active periclinal divisions occur in the prothallial cells of the venter region. The embryo continues division until it soon develops a foot, still in intimate contact with the prothallial tissue, as was the antecedent egg. A first leaf, stem apex and first root are then soon formed. The young embryo in about two weeks' time breaks through the surrounding prothallial tissue, which at first keeps pace with the enlargement of the embryo, becomes free on the underside of the prothallium and remains attached only by the foot. The stem and first leaf curve upward, the latter soon passing through the notch of the prothallium; the rapidly elongating root proceeds downward to establish contact with the substrate. This young sporophyte soon becomes independent of the prothallium on which it earlier depended for anchorage and nutrition.

In the preliminary work of this investigation it became necessary to perfect a technique for controlled fertilization in *Phlebodium aureum*. To do this it was of prime necessity to have available continuously an abundant supply of prothallia with mature antheridia and others bearing mature archegonia for mass production of embryos.

In attempts to satisfy these requirements, certain facts became obvious which seem worthy of addition to the story of the life-history of this species. These facts center around the following problems which are considered in some detail:

1. Growth of the prothallium
2. Opening of the antheridium
3. Opening of the archegonium
4. The fertilization process
5. Initiation and development of the embryo

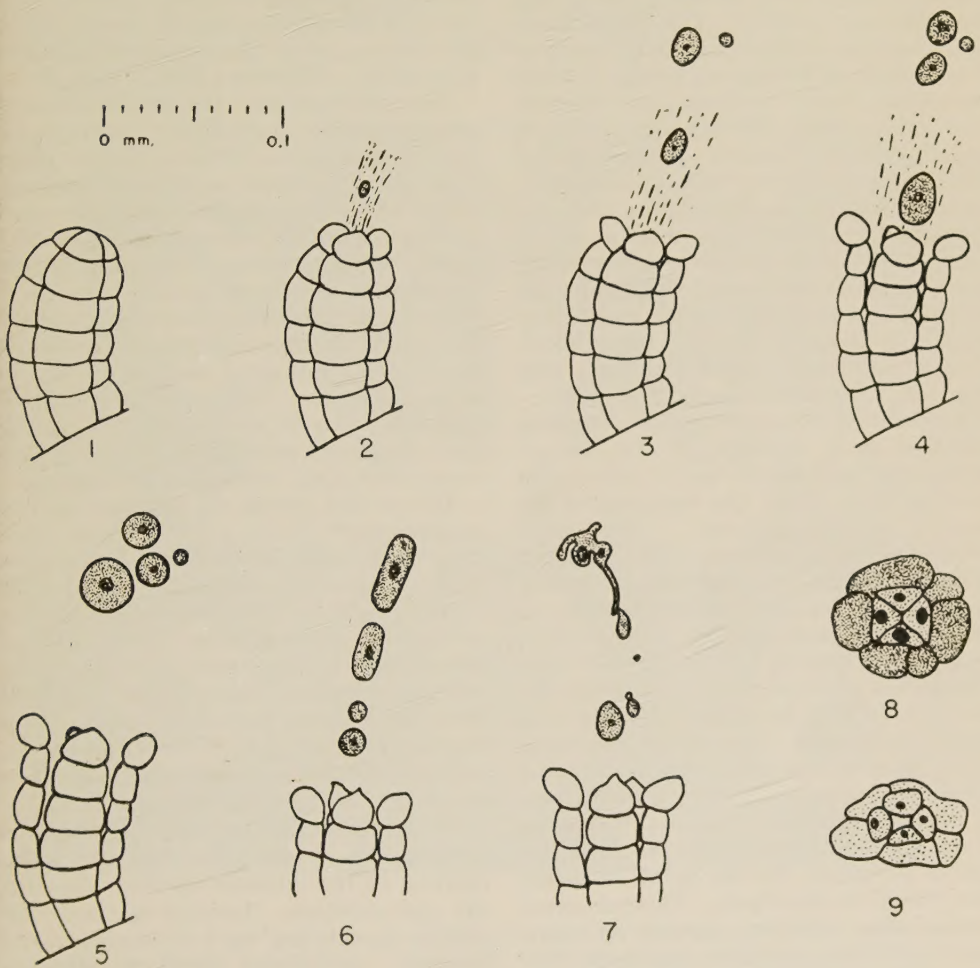
Growth of the Prothallium — Germination of the spores and immediately subsequent prothallial development were not considered in detail in these studies on *Phlebodium aureum*. Rather complete reports on this phase of the fern life-history are those of Hofmeister (1851), Strasburger (1869), Kny (1872), Bauke (1876), Prantl (1879, 1881), Goebel (1887), Campbell (1885, 1892), Conard (1908), and Orth (1936).

As mentioned earlier, soil-grown prothallia matured more rapidly and with an appearance of more vigor than did the cultures on nutrient agar. Scattered gametophytes matured more rapidly than those which grew in crowded condition, but even the plants grown most densely on soil did not remain indefinitely filamentous in form and antheridial in sexual maturity. The change-over from the initial antheridial state to the monoecious form was slower when crowded for space. Such growth characteristics were used to advantage throughout the investigation to provide prothallia of either antheridial or archegonial type. While it is true that during a part of the life of the gametophyte the plant is sufficiently monoecious for self-fertilization, antheridial prothallia were nearly always supplied in a quantity sufficient to provide an excess of sperms. Such prothallia were taken from the very dense agar cultures or from the younger, more crowded plants of the soil cultures.

In general the pattern of growth of prothallia on agar paralleled that of the soil-grown plants. The chief difference was noted in the slower growth and less vigorous development that characterized the agar cultures. The medium containing sucrose produced prothallia that early became monoecious, and many of the plants produced sporophytes, especially if they grew along the side of the flask where drops of condensed water were at times available for fertilization. An almost continuous supply of antheridial prothallia was provided by cultures grown on the medium without sucrose. Some of these cultures were planted so thickly with spores that a high percentage of the prothallia remained filamentous and antheridial for over a year during these studies. Scattered prothallia on the same medium developed eventually to the archegonial condition, although not much success was achieved with these plants in the production of embryos.

Antheridial plants were generally kept for a few days on moist paper after first being used. During this time more of

the organs matured, and the material was re-used to provide more sperms at later times. Many of the archegonial prothallia that at first failed to produce embryos were similarly successfully used if a week or so was allowed for maturity of more sex organs following the first immersion in water. Neither antheridial nor arche-



FIGS. 1-9 — Figs. 1-5. Successive stages in dehiscence of the archegonium in *Phlebodium aureum*, drawn from a motion picture sequence. Fig. 1. Archegonium enlarging in presence of water, prior to opening. Figs. 2-4. Stages in the discharge of the slime and globular masses of the disorganized canal contents. The last body (at Fig. 4) was seen in the picture to move slowly from the venter cavity and to come forth as the final in a series of discharges. The masses assume a rounded shape after a few minutes in the water. Apical cells bend outward and the four neck cell rows split apart to the fourth cell from the apex (Fig. 5). Fig. 6. Discharge of only slightly disorganized canal contents. Fig. 7. An advanced stage in canal cell disintegration. Fig. 8. Proximal neck cells (shown nucleated) tightly closed three days after fertilization. Deep staining of adjacent cells precedes meristematic activity. Fig. 9. Typical open canal 14 microns above venter cavity at narrowest point of passage in unfertilized archegonium. (Sections in Figs. 8, 9 are in a plane perpendicular to the axis of the archegonium.) Archegonia in Figs. 1-7 normally point downward on underside of the prothallium, and the curvature is away from the apical notch. The darker bodies in the masses of exudate in Figs. 3-7 are assumed to be nuclei, although no staining was used in verification. Scale is applicable to all figures.

gonial plants were used more than two or three times, largely because the supply of fresh material was generally abundant.

Opening of the Antheridium — The mechanism of antheridial dehiscence is capably presented by Schlumberger (1911) and Hartman (1931). The development and structure of the antheridium are presented in detail by Davie (1951). General agreement exists between the observations of Hartman and those of this study on *Phlebodium aureum*. The time required for the dehiscence of antheridia after immersion in water varies with the condition of the prothallia, usually ranging from 3 to 7 minutes for normal plants that have not been subjected to slight drying. In the latter condition, the opening may be instantaneous. On the other hand, some of the green, turgid antheridia may open after 10-15 minutes in the water.

The mass of sperms in the antheridium becomes more distinct as the mature antheridial cells absorb water prior to the opening (Fig. 10). The discharge of the sperms begins suddenly. The coiled bodies obviously escape under considerable internal pressure through an opening made by partial loosening of the cap cell from the adjacent ring cell. The process, as observed directly and as checked from the motion pictures, is completed rapidly, one sperm being forced out at a time in quick succession. The entire discharge is completed ordinarily in about two minutes, although frequently a sperm may fail to escape at all (Fig. 13). Activity of the sperm retained within the antheridium is permitted by the influx of water after escape of the others. These retained sperms often continue motion for more than two hours, gradually coming to rest.

The rounded, compact bodies of the still inactive sperms lie in a mass near the antheridium (Figs. 11, 12). In less than a minute, as a rule, and often within 30 to 40 seconds, the body expands slightly from the compact condition of discharge and assumes the loose, helical form of the swimming sperm.

Almost instantaneously and with marked rapidity the sperm moves off spirally through the water. Vigorous prothallia usually produce sperms which begin swimming with no preliminary movements. In less vigorous plants or those slightly dried, limited movements of the sperms are frequently perceptible. These can be detected at a magnification of 120×. Sperms from such antheridia show less subsequent vigor in movement. Activity of the cilia may be noted before the sperm body as a whole assumes movement (Atkinson, 1894).

Very infrequently do the discharged sperms remain completely inactive for more than a minute or so. In such cases they are subnormal in general activity. Antheridia which have previously lost considerable water often open rather normally and may discharge sperms that do not assume movement at all.

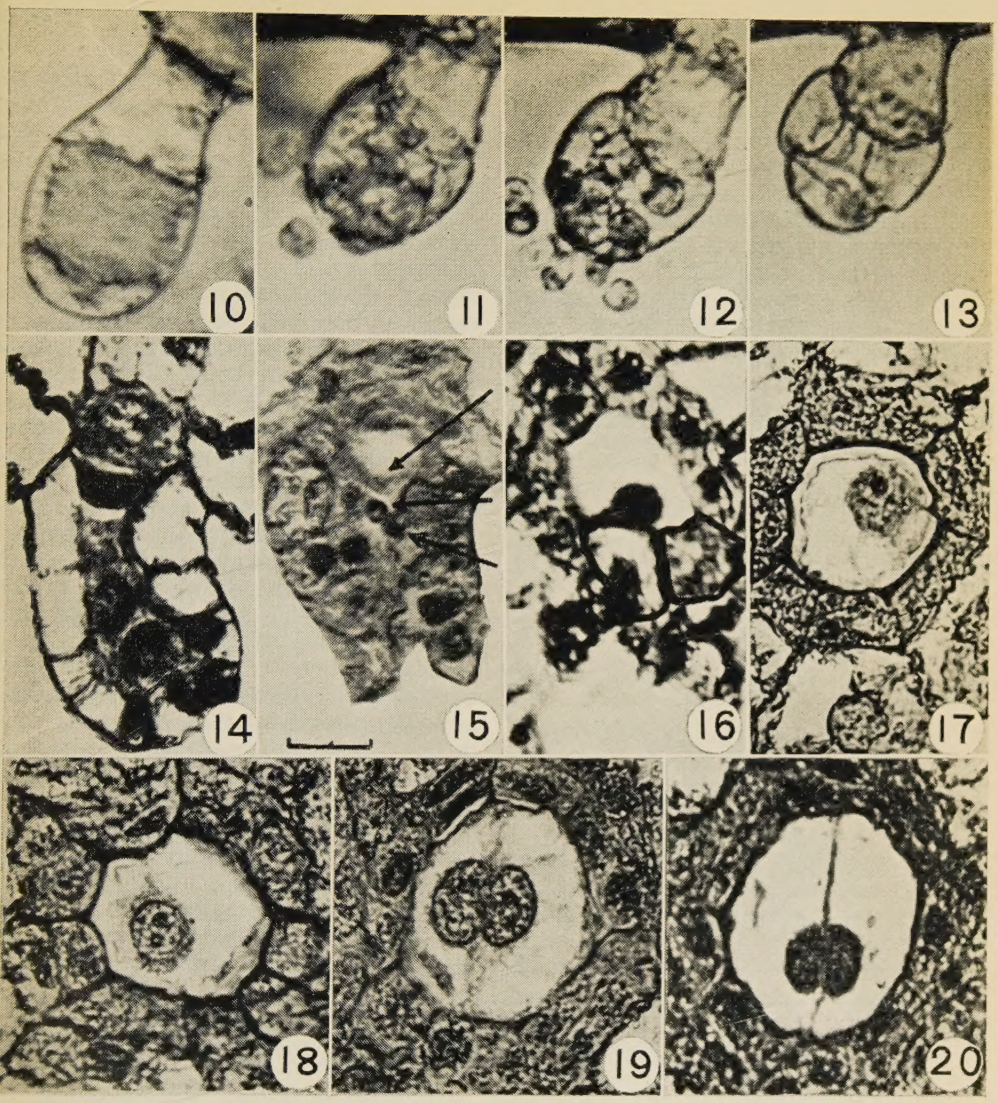
Rotary motion of the sperm is characteristic. The moving cilia are not seen distinctly at ordinary magnifications in living material. Their indistinct, blurred movement may be noted, however, in the case of sperms which are greatly slowed down after long periods of swarming.

The swarm period of sperms was not investigated. Buller (1900) found activity to continue for about 120 minutes under ordinary conditions. Dracinschi (1930) notes that the life span of some swimming sperms may extend to a maximum of two hours. The extended investigations of Pfeffer (1884) showed that the swarm period is affected by various concentrations of salts in solution, and Voegler (1891) found the period also dependent on temperature.

It is of passing interest that sperms were made immediately inactive in a solution of the nutrient medium used for the agar cultures. Activity was renewed quickly by diluting the fully concentrated medium. Antheridia failed to open in the same solution, but immediately opened in tap water subsequently added.

Swimming sperms are easily transferred with a medicine dropper to another prothallium and the region of opened archegonia. This facilitates immediate observation of the attraction of the sperms and the activity in the fertilization process.

Opening of the Archegonium — Archegonia may be considered mature when they open normally in the presence of water within an hour or so after immersion. Immature archegonia will not open



FIGS. 10-20 — Figs. 10-13. Stages in dehiscence of antheridium and release of mature sperms, enlarged from motion picture film. (Figs. 11-13 are of same organ.) Fig. 10. Imbibition of water by mature antheridium. Figs. 11, 12. Discharge of sperms from an antheridium. The tightly coiled sperms lie motionless in the water. Fig. 13. Three minutes later one sperm, unable to escape from the interior, is visible as a darker mass through the cap cell. Organs occupy natural position on ventral side of prothallium in rhizoid region. Fig. 14. L.S. of mature unopened archegonium, pointing away from apical notch. Nuclei of egg, ventral canal cell and neck canal cell are visible in interior. Fig. 15. Sperms (two lower arrows) are prevented from reaching egg by ventral canal cell (upper arrow) in mature archegonium prematurely opened with dissecting needle. Fig. 16. Heavily inked lines outline two neck cells in the plane of the archegonium axis, now tightly closed over the embryo two days after fertilization. Figs. 17-20. Cross-sections of young embryos from two to five days of age. Sections are tangential to the under surface of the prothallium and in a plane perpendicular to the main axis of the archegonium. The apical notch is directly to the left in the series. Enlargement of the embryo and division in the surrounding jacket cells are evident. Figs. 17, 18. Two and three-day-old embryos, respectively. Fig. 19. Five-day embryo with nuclear divisions completed and traces of the basal wall in formation. Fig. 20. Completed basal wall in five-day-old embryo. Scale in Fig. 15 is 20 μ in length and is directly applicable to Figs. 14-20. Dimensions of Figs. 10-13 are 1·1 $\times$ scale in Fig. 15.

naturally, even if longer exposed to the water. Overly mature or slightly dried organs open immediately on contact with the water or within a very few minutes thereafter. Outwardly, mature archegonia may be easily recognized by the full rows of four or five (and up to seven) well-developed neck cells (Figs. 1, 14). The apical portion of the structure when mature is somewhat enlarged and distended.

TABLE I — TIME FOR ARCHEGONIAL OPENING AFTER IMMERSION IN WATER

(*Figures are drawn from* 15 *observations*)

Minimum opening time	15 minutes*
Maximum time	82 ,,
Average of 15 observations	39 ,,
Openings in 10-19 minutes	2
Openings in 20-29 minutes	3
Openings in 30-39 minutes	6
Openings in 40-49 minutes	0
Openings over 50 minutes	4

*Apparently normal archegonia with fertile eggs have been noted to open in about five minutes. None of these was among the 15 cases on which these figures are based.

The series of observations was made on different days, entirely at random, and is believed to be representative for plants of normal turgidity, direct from soil cultures. All observations were made at room temperature. Tap water was used throughout.

Previously opened and unfertilized archegonia are easily distinguished by the brownish color of the inner walls of the neck cells and of the venter and by the slightly shrunken character of the latter. Just apically to the desiccated archegonia are found ordinarily the most mature, unopened organs. The latter archegonia, having full size and manifesting general turgidity, may well be expected to open normally within a period ranging from five minutes to one hour. Table I gives the average time as 39 minutes for opening of archegonia on 15 sample prothallia. Archegonia on prothallia which bear embryos do not, as a rule, open even after prolonged immersion (Table II).

Impending opening of water-covered archegonia is at first evident in the further enlargement of the distal portion of the archegonium. The neck cell walls become clearly outlined, and the swollen apex becomes rather transparent. The distension apparently results from an internal pressure created by the neck canal contents. Often slight movement of hyaline masses may be seen as they are forced apically before any exterior parting of the four apical cells actually takes place. Such movement was noted by Campbell (1887).

Separation of the apical cells is at first evident in a tiny stream of mucilaginous material issuing from the exact apex. No

TABLE II — RESULTS OF IMMERSION IN WATER OF PROTHALLIA BEARING ARCHEGONIA AND YOUNG EMBRYOS

No. of Prothallia	Age embryo	Hours in Water	Archegonial Response
2	8 days	2	No opening
1	9 ,,	3	,,
1 (2 embryos)	9 ,,	3	One opening*
1 (2 embryos)	9 ,,	3	No opening
1	10 ,,	3	,,
1	12 ,,	2	,,

*Discharge of canal contents was not observed. It was noted at the time that discharge was incomplete, and evidently lacked the usual forceful projection of the slime bodies, leaving the exudate lying near the mouth of the archegonium. Rupture of the mouth cells was irregular, having the appearance of tissue of subnormal turgidity. No sperms were supplied as a test for attraction and none was observed naturally present in the water. (Observations were made in March.)

All prothallia selected bore from four to six and up to many archegonia of the size and shape of normally virile organs. Slight desiccation characterized the over-all appearance of most of the archegonia.

sharp movement is seen to initiate the flow, which proceeds slowly for a few seconds and then sharply increases in amount as the fissure rapidly enlarges. A sizable mass of rounded, colorless material is then forcibly ejected from the opening (Fig. 2), the stronger projection being evident in archegonia of vigorous, green prothallia. This mass is thrown normally at a distance of about one archegonium length away from the mouth.

A further flow of the mucilaginous substance follows. Within a few seconds another globular mass is thrown from the now widened archegonium mouth. It is followed by a decreasing flow of slime. As in the case of the first globular body, the material appears under considerable pressure, and the movement, as it leaves the mouth, is in most cases practically instantaneous and with sufficient strength to carry the exudate 50 to 100 microns away.

A third body is usually expelled, but its presence and size are dependent on the state of disintegration of the canal cells prior to the discharge. The uppermost neck cells of the archegonium meanwhile turn outward, often visibly snapping sharply to a new position. Concomitantly with this outward turning, the four rows of neck cells split apart, usually down to the third or the fourth cell (further if the neck rows have more than five cells, as in Figs. 4, 5).

Characteristically in normally turgid and mature archegonia a fourth hyaline mass, the largest of those discharged, is now thrown forth, usually with a little less vigor than the first. The slime flow is generally not further noted. The canal is now completely open all the way to the exposed venter, for this latter mass seems to be the remnant of the ventral canal cell with an obvious nucleus (Fig. 4). The elapsed time for the complete process from the first evidence of slime flow is ordinarily no longer than a minute and one-half.

As a rule in *Phlebodium aureum* the most apical of the neck cells are not broken off during the opening. Hofmeister (1851) and Campbell (1918) are among those who called attention to the throwing off of these cells during the opening of the archegonium in other species. In plants having a low water content this is almost always the case, for the addition of water rapidly hydrates the intercellular binding material. The apical cells, as well as adjoining ones, are under more obvious tension and the neck rows literally collapse in the more extreme cases. The incidental discharge is not complete, as the dehiscence occurs before the water can be adequately absorbed into the interior of the archegonium to insure a forcible expulsion of the canal contents and a consequently fully opened canal. The slime and incidental hyaline bodies, variously disorganized, are left at or near the mouth of the archegonium instead of being thrown clear as is accomplished in the usual case (Figs. 2-5).

In *Phlebodium aureum* the slime diffuses away considerably within a few minutes after the opening, and the globular masses still in the vicinity assume a nearly spherical form (Fig. 5). Variations in form and degree of disintegration of the freshly discharged neck canal contents are shown in Figs. 4, 6, and 7.

The Fertilization Process — The arrival of sperms before the mouth of the newly opened archegonium is generally not immediate, unless they are already in the vicinity through attraction from a previously opened archegonium. The first sperms to arrive seem to be attracted to the region by a diffusion substance that in undisturbed water extends outward in a funnel-shaped expanse from the archegonium mouth.

Sperms crossing this diffusion area react violently and change direction chemotactically toward the region of attraction. This compares with the behavior of sperms that Hanstein (1865) described as being grasped by a whirlpool before the archegonium mouth. They soon become oriented anteriorly toward the open canal, and usually return quickly if they chance to swim momentarily from the central attraction area. A few of the less active sperms may be attracted by the canal exudate. Some of them often become temporarily entangled in the plasmic substance, where they remain in continual rotary motion for some time.

As observed in this species, sperms do not, as a rule, enter the freshly opened canal immediately on arrival. They circle about, ultimately pointing, as they rotate, toward the region of the mouth. It seems that the opened archegonium exudes substances which at first strongly repel the sperms that draw near. Their behavior indicates that sperms react to an optimum concentration of such chemicals, and that they enter the canal only after a few minutes' delay. Meanwhile the diffusion of water into the opened canal possibly dilutes the substances toward an optimum for positive stimulation.

Pfeffer (1884) and Buller (1900) found sperms repulsed at higher concentrations of various test substances, including malic acid and its salts. Buller, with the use of capillary tubes containing the test attraction substances, found sperms repelled from the mouth of a tube having a high concentration. The sperms moved to a zone at which the substance no longer repelled and there they resumed normal motion. They gradually approached and entered the tube as the diffusion of the substances reduced the concentration to one of attraction instead of repulsion.

Within three to five minutes after the discharge of the archegonial contents, the sperms, which may have oriented themselves before the archegonium mouth, draw nearer and soon enter the canal. They are not generally retarded by the slime so often mentioned in descriptions of the process for other species. Movement down the canal is gradual in the usual case, although active rotary motion continues in the more confined space.

The canal of the archegonium is sufficiently wide in most cases for the sperms to proceed without the elongation from the helical form mentioned freely in other species by Strasburger (1869), Campbell (1887), Bauke (1876), Atkinson (1894), Shaw (1898), and Atkinson (1943). Campbell contended that many of the sperms were unable to enter the archegonium at all, and that only a fresh and active antherozoid could make its way to the germ cell. The narrowest part of the canal is that between the four proximal neck cells just above the venter cavity. Even these appear to furnish a passageway that but little hampers the sperm. The opening in living archegonia seems to be of a diameter very nearly that of the coiled body of the sperm. Only in obviously exceptional cases is the passageway much larger.

Once in the venter, freedom of motion is permitted to the sperm by a space above the egg that ranges in width from three to four diameters of the coiled sperm body and in height somewhat less. The discharged ventral canal cell previously occupied the cavity. (Fig. 15 shows sperms that were prevented from reaching the egg by the retained ventral canal cell.)

Accelerated rotary motion is characteristic of sperms reaching the venter. Stronger chemical stimulus than before seems to be exerted upon them. Motion at first is vigorous and at random, but soon the sperm ceases side-to-side gyrations and stands with the flagellate (anterior) end against the egg until movement gradually stops, in times ranging from 4 to 20 minutes in this species. After that time, the form of the sperm can usually no longer be recognized.

The motion of the sperm in the ventral cavity was observed by Hofmeister (1851) to be about seven minutes. Strasburger (1869) described cessation of motion in times ranging from three to ten minutes. Voegler (1891) described a case of movement lasting 45 minutes, while Campbell (1892) no longer saw activity after three to four minutes. These variations in time were seen in species other than *Phlebodium aureum.*

The earliest time noted for entrance of a sperm into the venter after the starting of the discharge was two minutes in *Phlebodium aureum.* This was accomplished by a vigorous sperm that had just arrived in the vicinity, and its forward momentum was scarcely impeded by the canal itself. Normally, more time elapses before gradual entrance into the canal and the slow progression down the passage. Not uncommonly does an active sperm pass less active ones *en route.* Sperms once in the venter may come back out, but the occurrence is infrequently observed. Those that have entered only the canal region frequently make their

way out. Such occurrence was reported by Pfeffer (1884), Shaw (1898), and Thom (1899).

Rarely do more than three to five sperms occupy the ventral cavity at one time. Hofmeister (1851) observed three sperms in the cavity simultaneously, and Strasburger (1869) described as many as four to five present at once. Many more, if present, may arrange themselves outside so that they point toward the archegonium mouth where they remain. They gradually slow down, elongate and become motionless, usually well within one hour. This forms the characteristic funnel-shaped mass before the archegonium mouth early pictured by Hanstein (1865) and subsequently by Strasburger (1869), Shaw (1898), and Conard (1908).

In time the discharged bodies lose completely any attracting quality they may have earlier possessed. The only remaining attraction is by soluble substances diffusing from the neck canal. Only sperms passing near the mouth of the canal show altered direction of movement. Rarely is an archegonium attractive after 45 minutes following the opening. Sperms already present, however, may remain longer with gradually diminishing movement.

The cytology of the sperm after entering the egg and the actual nuclear fusion was not made a part of this work. Campbell (1892), Shaw (1898), Mottier (1904), and Yamanouchi (1908), among others, have followed the process in great detail from killed and fixed material.

Initiation and Development of the Embryo — Successful fertilization may be recognized with certainty in living prothallia, often within 24 hours after entrance of the sperms. The neck cells remain green, with only the upper inner walls tending toward browned or darkened coloration; and the proximal neck cells become drawn tightly together (Figs. 8, 16). In the most vigorous plants, beginnings of cell enlargement and division in the region of the venter are evident within 48 hours. Stained sections show an enlarged egg and distinctly deeper staining in the adjacent prothallial cells as characteristics of fertilization.

Most prothallia observed in *Phlebodium aureum* bear only one embryo. However, the occurrence of more than one on a given prothallium (resulting generally from simultaneous fertilization) is rather frequent in vigorously growing plants. As a rule, only one sporophyte on each prothallium continues indefinite development. In a given number of prothallia obviously suitable for successful fertilization, there are generally some which do not produce embryos. Table III sum-

TABLE III —NUMBER OF SUCCESSFUL FERTILIZATIONS IN OBSERVED PROTHALLIA (MARCH-APRIL)

Observation No.	Condition of Prothallia	No. of Prothallia	No. of Prothallia Producing Embryos
1	Mature; old	10	3
2	Mature; old	13	3
3	Young; vigorous	9	6
4	Re-used†	5	4
5	Vigorous	9	9§
6	Re-used*	13	2
7	Young; vigorous	15	10
8	Re-used*	7	5
9	Re-used*	6	4
	Total prothallia used	87	
	Total prothallia-bearing embryos	46	

*Re-used prothallia were previously water-immersed for possible fertilization without positive results. Such prothallia, kept on damp paper and usually with soil retained by the rhizoids, were available for further use a few days later following maturity of more archegonia.

†Re-used four days after the first immersion.

§Five of the prothallia bore either two or three embryos.

TABLE IV — FIRST DIVISIONS OF FERN EMBRYOS

Investigator	Year	Fern	Division first recorded
Hanstein	1865	*Marsilea*	12 hrs. after fertilization*
Campbell	1887	*Onoclea struthiopteris*	24 hrs. after fertilization
do	1895	*Marsilea*	1 hr. after fertilization
do	1895	*Pilularia*	3 hrs. after fertilization
Shaw	1898	*Onoclea struthiopteris*	9-10 days after fertilization
		Onoclea sensibilis	8-10 days after fertilization
Conard	1908	Hay-scented fern (*Dennstaedtia punctilobula*)	7 days after flooding cultures†
Yamanouchi	1908	*Nephrodium molle*	1 week after entrance of sperm
The present study	—	*Phlebodium aureum*	5 days after fertilization

* Later noted by Shaw (1898) to be erroneous.

†" Fertilized eggs were found about seven days after my cultures were flooded with water", Conard notes. It is assumed from his further discussion that successful fertilization was indicated by cleavage.

marizes the number of embryos noted on 87 prothallia which had been subjected to conditions favorable for fertilization. As many as five embryos have been observed on a single prothallium in this species.

Unfertilized eggs and the inner walls of the archegonium venter and canal cells usually show a brown coloration within 24 hours even though sperms actually reached the venter and the egg cell. The four proximal neck cells do not draw tightly together and their reaction to staining is limited (Fig. 9).

Within 48 to 72 hours the fertilized egg cell in a living prothallium is enlarged, appearing as an almost transparent mass when dissected. The cells of the venter meanwhile begin an accelerated proliferation. The entire region of the embryo can be told at a glance under magnifications above 70× with transmitted light. Conard (1908) noted 16-day old embryos of *Dennstaedtia punctilobula* through a hand lens. Embryos of that age in *Phlebodium aureum* are readily seen with the naked eye.

The first cell division of the embryo occurs in about five days or slightly longer, depending on the vigor of the prothallium. Completed divisions with a cell wall, beginning divisions, and fertilized eggs without evidences of division were all found in sections of prothallia fixed five days after entrance of the sperms. Fertilized eggs at this age are clearly distinguishable from unfertilized ones through the size and staining qualities of the former. No divisions were found in embryos younger than five days. Table IV summarizes the times of first divisions in some different species of ferns as found in past investigations.

The first division wall in *Phlebodium aureum* is rather uniformly parallel to the axis of the archegonium and at right angles to the long axis of the prothallium. This follows generally the findings of most workers in the higher ferns. Hofmeister (1851), Bauke (1876), Campbell (1887), Atkinson (1894), and Conard (1908) report similar orientations of the first wall in other species. Cross (1931) in *Osmunda cinnamomea* found the first division wall parallel to the axis of the archegonium and parallel to or at a variable angle with the anterior-posterior axis of the prothallium, instead of perpendicular to such axis.

Stages in development of the fertilized egg, including establishment of the first division wall, are shown in Figs. 17-20.

The enlarging prothallial cells surrounding the embryo (the calyptra) form a mantle that keeps pace with the growth of the embryo for about 16 days following fertilization. The first leaf then breaks through, followed almost immediately by the endogenously formed first root.

Only one sporophyte has been observed to continue toward full development on any given prothallium in these investiga-

tions on *Phlebodium aureum*. A sectioned prothallium bearing a 24-day old sporophyte shows that a second embryo on the same plant was inhibited in development at the eight-celled stage. This arrested growth appears to result from the presence of the more vigorous sporophyte. Removal of a 20-day old sporophyte, with well-developed first leaf and root, permitted a nearly inhibited embryo to resume development. In both cases here referred to, the two embryos on the same prothallium resulted from fertilizations at the same time, and were observed to be about the same size on the third day after fertilization was permitted. Within a very few days one of the embryos showed increasingly rapid enlargement while the other ceased abruptly to gain in size. Also noted was a continued development of two adjacent embryos which were separated by a cut in the prothallium. Development of both was continuing rather normally at the time of appearance of the third leaf.

Discussion

In the course of perfecting techniques for culture of prothallia in *Phlebodium aureum* and subsequent controlled fertilization in this species, certain aspects of the life-history proved to be worth some detailed comment. These facts have been considered earlier *per se*. The present section of this paper relates these observations on *Phlebodium aureum* with findings previously reported for other species, which collectively form the essence of the current knowledge of the fern life-cycle.

Spores of *Phlebodium aureum* were found to germinate best when freshly shed from the sporangia. Spores stored in the refrigerator for a few weeks showed less viability, both in number of spores germinating and in the vigor of the prothallia produced. No statistical study was made, but the general tendency toward lowered viability was evident in the resultant cultures. In one instance, spores collected in January were found to be only slightly viable in March agar cultures. No efforts were made to study the effects of lyophilizing or quick freezing of spores, since abundant quantities were always available.

Varied findings on viability of fern spores have been reported. Campbell (1885) noted that spores in general have a high vitality. Spores of *Woodsia ilvensis* over a year old were found to germinate. Atkinson (1894) contended that several months' dormancy is required for most ferns, the Hymenophyllaceae and Osmundaceae excepted, and that some species are viable for several years. Benedict (1939) found germination in most species best when the spores were sown immediately after ripening, although some types were found viable for months. More precise tests must be made to establish the extent of spore viability in different species and under different conditions.

The works of Bauke (1876), Prantl (1879, 1881), Heim (1896), Nagai (1914), Orth (1936), and such recent workers as Hurel-Py (1950, and earlier) and Sussountzov (1948, and later papers) using *in vitro* cultures, have shown the plasticity of the fern prothallium. It responds readily to environmental influences of various kinds. These earlier reports indicated that to have a continuous supply of antheridia one had only to plant spores densely on the substrate selected. By contrast, the same earlier findings indicated that prothallia would rather quickly develop to the archegonial condition if the spores were planted sparsely, or if the young prothallia were thinned to remove the effects of competition. Such growth characteristics also prevail in *Phlebodium aureum*, although an abundance of prothallia made unnecessary the use of the thinning technique.

Soil-grown prothallia generally reached maturity before those grown on agar. Both kinds were satisfactory for most experimental uses in this study. In the past, prothallia have generally been grown on soil or some related form of substrate. The sterile nutrient agar technique for culture is more recent and has been found quite successful (Hires, 1940; Nebel, 1946; Hurel-Py, 1950; Morel and Wetmore, 1951). Varied refinements for the culture medium, as suggested in the recent

works of Hurel-Py and Soussountzov, have not yet been tried in the present studies.

Past investigators have widely used fern prothallia that have been slightly dried, for their studies on fertilization. The general practice was simply to refrain from watering the cultures for a day (or longer in some cases) prior to observation. The effect was, as known from the time of Hofmeister (1851), a more rapid opening of both antheridia and archegonia. Greater numbers of sex organs became mature and were held in readiness for more extensive observations in shorter time upon the application of water. Pfeffer (1884), Campbell (1887, 1918), Voegler (1891), and Bower (1908) have recorded the advantages of the drier plants for more rapid opening of the sex organs. The same response is found in *Phlebodium aureum*, although no real use of the technique was made. It early became evident that certain erroneous conclusions might result from using desiccated prothallia of this species. These conclusions seem worthy of comment.

While antheridia of *Phlebodium aureum* tend to open normally in three to seven minutes, organs on drier prothallia may opon almost upon contact with the water. Atkinson (1894) noted that antheridia in general opened in a period of five to twenty minutes. Pfeffer (1884) found that antheridia of somewhat dried prothallia opened better than others and in a time range of five to ten minutes. Most of the prothallia of *Phlebodium aureum* used in the determination of the time for dehiscence were filamentous forms from the nutrient agar cultures. They were consequently a little drier than the normally watered soil-grown plants.

Archegonia on prothallia grown for several months on agar are also generally somewhat drier than those on soil-grown prothallia. Such organs may break open almost immediately upon contact with water. Less desiccated archegonia from fresher cultures, or from soil only slightly watered, open less rapidly, yet predominantly within a few minutes if at all mature. In summary, it may be said for *Phlebodium aureum* that the use of dried prothallia presents a fertilization picture that is abnormal in some or all of the following points:

1. Dehiscence of the archegonium is more rapid.
2. Throwing off of the apical neck cells frequently occurs.
3. The rows of neck cells often part haphazardly, rather than in an orderly, away-from-the-canal, four-directional dehiscence.
4. The slime and globular masses of the canal contents are not thrown free and are left near or within the archegonium mouth.
5. The canal is likely to remain obstructed with residual slime.
6. This slime obstructs the movement of sperms and prevents their entering the canal and proceeding to the venter and the egg, the diffusion center of the attracting substance.
7. Successful fertilizations and resultant embryos are markedly fewer.

Much attention in past reports of fertilization in ferns has centered around the evident difficulties met by the sperm in gaining passage through the discharged slime into the archegonium mouth and ultimately into the egg-containing venter. Complete blocking of the entrance has been described. Strasburger (1869) found sperms of *Ceratopteris* and *Pteris* hindered by the slime in their movement down the canal, as well as in the region outside the archegonium mouth. Atkinson (1894) described the granular slimy mass of disorganized canal cells as protruding from the opened archegonium and filling the canal. Shaw (1898) observed in *Onoclea struthiopteris* that the mucilage seemed to be more dense in the canal. Yamanouchi (1908) noted in *Nephrodium molle* that some of the disorganized material remained in the neck and venter region of the archegonium, even after it had opened and become accessible to water. Campbell (1892) found that sperms of *Onoclea struthiopteris* entered the opened archegonium in a few minutes, not much impeded by the exuded mucilaginous substance.

In *Phlebodium aureum*, as observed in these studies, the normal disposition of the discharged canal cells and the attendant mucilaginous substance is at some

distance from the archegonium mouth. As a consequence, the canal is effectively open throughout its entire length to the egg cell in the venter. The ability of sperms to move freely in the canal, and in fact often to enter with almost unchanged velocity, is the clearest evidence that the passageway is normally free of obstruction noted in other species and attributed to residual slime.

The three or four successive and explosive discharges of the canal contents are a striking feature of the opening of the archegonium in *Phlebodium aureum.* Little reference to a similar procedure appears in reports on other species.

It is true that for a time some attraction is offered by the discharged contents of the archegonium, and some sperms often become more or less entangled in the exudate (a few are unable to free themselves at all). Yet this is not a significant part of the fertilization picture as observed in *Phlebodium aureum.* The nearest parallel to the effects of slime on fertilization as noted in other species is that found in the use of desiccated prothallia. Then, as has already been noted for *Phlebodium aureum,* the neck cells, when covered with water, pull apart immediately or within a few minutes. This does not permit accumulation of sufficient internal pressure to carry the slime and disorganized canal cells away from the mouth of the archegonium.

Opened archegonia which do not result in successful fertilizations, whether sperms have entered the venter or not, usually begin to display brownish coloration of the inner walls of the neck cells and the venter in less than 24 hours after dehiscence. Successful impregnations are distinguished in *Phlebodium aureum* by the fact that both neck cells and venter remain green. Only the inner walls of the upper neck cells gradually show brownish coloration.

Hanstein (1865) in observations on *Marsilea* assumed that fertilized archegonia quickly turned brown and the unfertilized remained green. He noted, however, that the venters of some of the archegonia that later produced embryos remained green. The fertilization became evident through the enlarging embryo. Hofmeister (1851) found that the walls of the unfertilized archegonia and the cavity of the venter brown throughout, and that only the unclosed portion of the canal in fertilized archegonia turned brown. This is the case in *Phlebodium aureum.* Kny (1872) described and showed in colored plates the brown cell walls of the unfertilized archegonium in *Osmunda regalis,* and Bauke (1876) pointed out that brown inner neck walls in *Pteris aquilina* indicate failure of fertilization. Campbell (1918) contended that the walls turn dark brown almost immediately in fertilized archegonia.

The immediate closing of the canal by the proximal neck cells was noted by Hofmeister (1851) and by many workers since that time. Bauke (1876) called the closing of the canal not a simple mechanical action but a growth phenomenon immediately attending fertilization. Shaw (1898) and Mottier (1904) agreed that the closure occurred in about 30 minutes after fertilization. No actual time was determined in these studies for *Phlebodium aureum,* but the closure is readily evident in living material after 24 hours.

The number of prothallia which produce one or more embryos is perhaps in the main smaller than the number which do not bear embryos, even though conditions for fertilizations are obviously present. The rather high incidence of fertilizations shown in certain different observations in Table III is believed to be near the maximum likely to occur under favorable conditions. Such conditions include proper maturity and vigor of both male and female plants. Often not a single embryo has been found in dozens of prothallia in *Phlebodium aureum,* although no reason was immediately apparent which might account for the failure to produce embryos.

Hofmeister (1851) noted that barely one prothallium in ten produced a young sporophyte. Atkinson (1894) assumed from general observations that the large majority of prothallia perish without development of a new plant, even though fertilization has taken place. Conard (1908) noted that about half of the prothallia used in observations on *Denns-*

taedtia punctilobula produced embryos. Hoyt (1910) found 37 fusions in 97 observed archegonia on 59 prothallia.

The number of embryos produced on a single prothallium in *Phlebodium aureum* is quite comparable to that of findings reported in other species. Hofmeister (1851) observed that only one embryo (with rarest exceptions) developed into a mature sporophyte. Campbell (1885) generalized that several archegonia might be impregnated on a given prothallium, but that only one developed to maturity. Atkinson (1893) reported two embryos on one prothallium of *Adiantum cuneatum*. He found normal development in the young specimens, as examined from sections after root and cotyledon development, and he considered them capable of independent existence. More recently Haupt (1940) concluded that in *Angiopteris evecta* never more than one fertilized egg was ultimately functional on each prothallium.

The first division wall as found in *Phlebodium aureum* is generally parallel with the axis of the archegonium and at right angles to the long axis of the prothallium. This conforms well with the findings of other workers in different species of the higher ferns. The time for first division after fertilization in this species (about five days) is compared with times found for other species in Table IV.

In substance the findings reported for these investigations are refinements in detail rather than changes in the fundamental concept of the fern life-history. The necessity for production of embryos in quantity also forced consideration of a timing of events in order that the proper sequence could be effected at any time.

Summary

This report presents the essential features of the prothallial phase of the life-history of *Phlebodium aureum*. It is based mainly on information gained in the development of techniques intended to supply material for experimental work on the fern embryo.

Spores were germinated on both soil and nutrient agar media, and the growth of prothallia under the different conditions is considered.

The opening of the sex organs is described in some detail. Observations were supplemented and checked by motion picture studies.

Antheridia open abruptly within a few minutes in the presence of water, and the sperms are discharged one by one. The sperms become active and swim actively about after a few seconds of contact with the water.

The opening of the archegonium is characterized by the successive and explosive discharges of two to four globular remnants of the disorganized canal cells and attendant slime, effectively opening the canal. The last globular mass is recognized as the contents of the nucleated ventral canal cell, intact or somewhat disorganized.

Sperms are chemotactically directed to the open (and now empty) neck canal, at first by the remains of the extruded materials and later by an attracting substance having as its center the egg itself. The sperms enter the opened archegonium after a slight delay, essentially not retarded in movement by the discharged slime, and one sperm fuses with the egg cell within a few minutes.

The first division of the fertilized egg takes place about five days after impregnation, and is characteristically parallel to the axis of the archegonium and at right angles to the long axis of the prothallium.

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THE DEVELOPMENT OF THE EMBRYO OF *PHLEBODIUM AUREUM* J. SM.

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Introduction

It became evident early in the present series of investigations on morphogenetic factors underlying embryogeny in the fern *Phlebodium aureum* J. Sm. (*Polypodium aureum* L.) that a connected and detailed descriptive account of the development of the young embryo in this species must be obtained. This account is provided herewith. The subsequent experimental studies will appear in another publication.

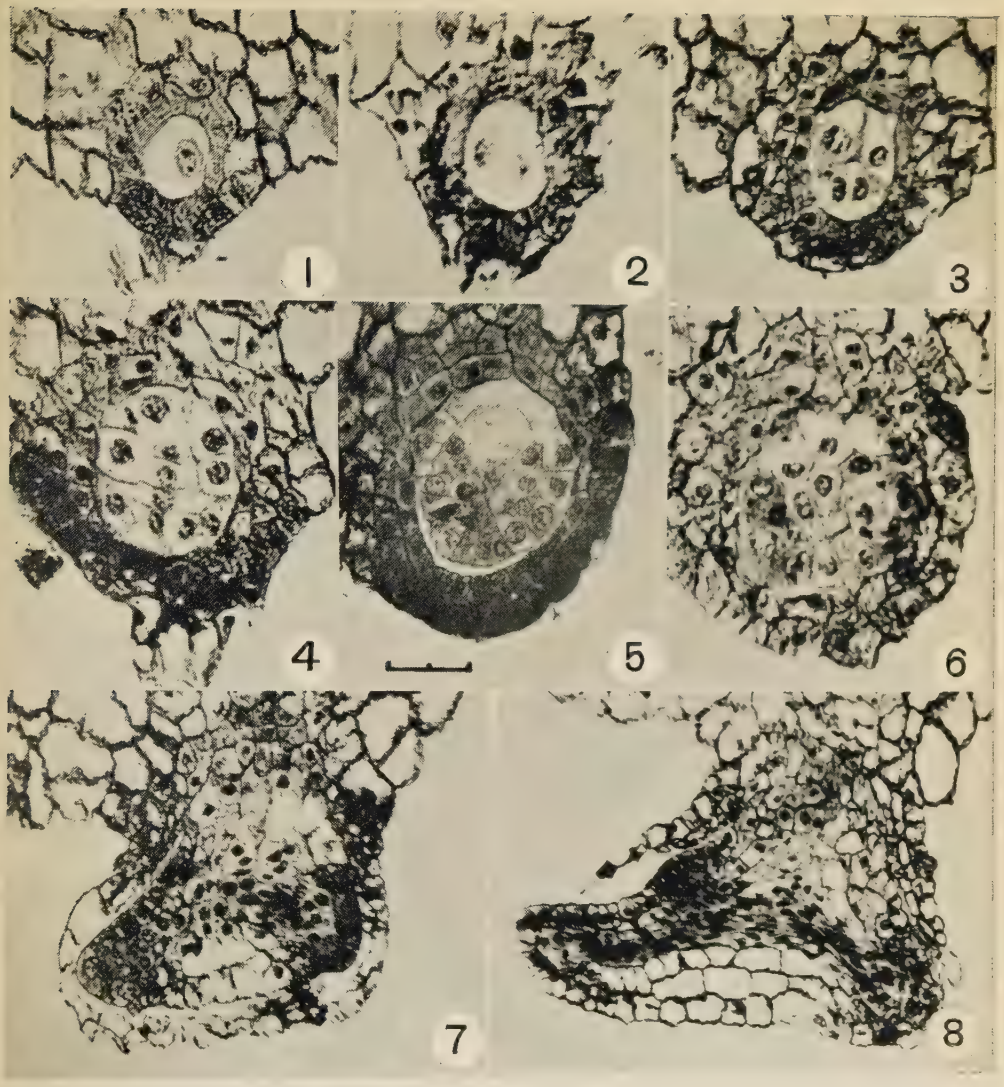
The present paper follows the development of the embryo from the time of first division of the fertilized egg through the early stages of development, including the appearance of the first organs or appendages. The time for such development is about 16 days, and the end of the period is characterized by the emergence of the leaf and root of the young sporophyte from the previously encapsulating prothallial tissue.

While study was given to all age phases, special attention was directed to multicellular stages, since only in these were morphogenetic variations accented significantly. Comparisons in the earlier stages of division were not immediately feasible.

The age of the embryo in this paper is arbitrarily set as the number of days following the opening of the archegonium (Ward, 1954), which permits sperms to enter the neck canal and set the stage for fertilization. Undoubtedly some variation in time exists between the passage of the sperm into the canal and the actual fusion of the sperm and egg nuclei. Yet, as the later stages of sperm activity are not visible in the venter of the archegonium, and the existence of the embryo is soon recognizable through the prothallial tissues, an arbitrary age is recorded from the last fixed event, the opening of the archegonium. Comparatively, the totals must be of value, and the variation in the pre-fertilization events must be relatively insignificant in view of the usual speed of penetration of the neck canal by sperms and the reasonably constant appearance of the first wall in the fertilized egg (Ward, 1954).

The calyptra is here assumed to mean the envelope of tissue that surrounds the young embryo in the early stages of development. The egg, at the time of fertilization, is enclosed by the wall of the venter, the base of the archegonium which lies within the prothallium. This venter wall is not dissimilar to the surrounding prothallial tissue. Following fertilization and before the first division of the young embryo, at five days of age, vigorous meristematic activity in these cells begins elaboration of the more complex embryonic envelope, a structure that covers the young embryo completely for about two weeks of growth and often attains a thickness of two or more cell layers (Figs. 1-8). To this thickened wall, Campbell (1892, 1905) transferred the term *calyptra* from the mosses, where it was earlier and widely used from the time of Hofmeister (1851) and his followers. In the ferns, *calyptra* is in general use (e.g. Conard, 1908; Smith, 1938) and the cells composing it are also commonly referred to as *jacket cells*.

The half of the embryo toward the apical notch following the first division was called by Vouk (1877), Leitgeb (1878), and Goebel (1887) the "epibasal" half, while the other portion was referred to as the "hypobasal" one. In this paper, the areas are simply called anterior and posterior, respectively, since the disposition of the two halves is not



FIGS. 1-8 — Stages in development of the embryo of *Phlebodium aureum*. Views are in a plane passing through the axis of the archegonium and parallel to the anterior-posterior axis of the prothallium in approximate position of natural growth. Apical notch is to the left in all figures. Fig. 1. Three-day-old embryo showing meristematic activity in surrounding calyptra. Fig. 2. Basal wall in formation in plane perpendicular to page; nucleus has completed division at 5 days. Anterior half of embryo is to the left; posterior half is shown at right. Fig. 3. Developing embryo just beyond the octant stage. Fig. 4. Cells in upper part of the embryo are enlarging to form the foot. Fig. 5. Leaf initials are evident at lower left at 11 days. Fig. 6. Leaf projects forward at lower left. Not clearly visible in photograph are endogenous root initials at lower right, just within the border cells of the 12-day-old embryo. Fig. 7. Stem initials are evident at the left, about midway between leaf apex and extremity of foot in 14-day-old embryo. Vascular strands are in formation toward stem area as a branch from leaf-root vascular axis. Pressure from enlarged leaf is beginning to rupture calyptra (lower left). Fig. 8. Further development of 16-day-old embryo with root now free from calyptra.

Scale in Fig. 5 is 50 μ in length and directly applicable to Figs. 1-6. Dimensions in Figs. 7 and 8 are, respectively, $1\frac{1}{2}\times$ and $2\times$ the scale in Fig. 5.

necessarily nor usually one above the other (Figs. 12-14).

Materials and Methods

Prothallia of *Phlebodium aureum* were grown as described previously (Ward, 1954). Fertilization was facilitated by immersion of the plants in water for an average time of two hours. The prothallia were then replaced on soil or sand. They were examined for developing embryos daily from the second day following fertilization, and the prothallia bearing the embryos of the desired age were fixed and sectioned. Embryos of ages ranging from 2 to more than 20 days were sectioned and studied under the microscope.

Newly fertilized eggs were stained for observation by Sudan IV and Lugol's iodine in addition to the widely used iron alum-haematoxylin method.

Observations and Results

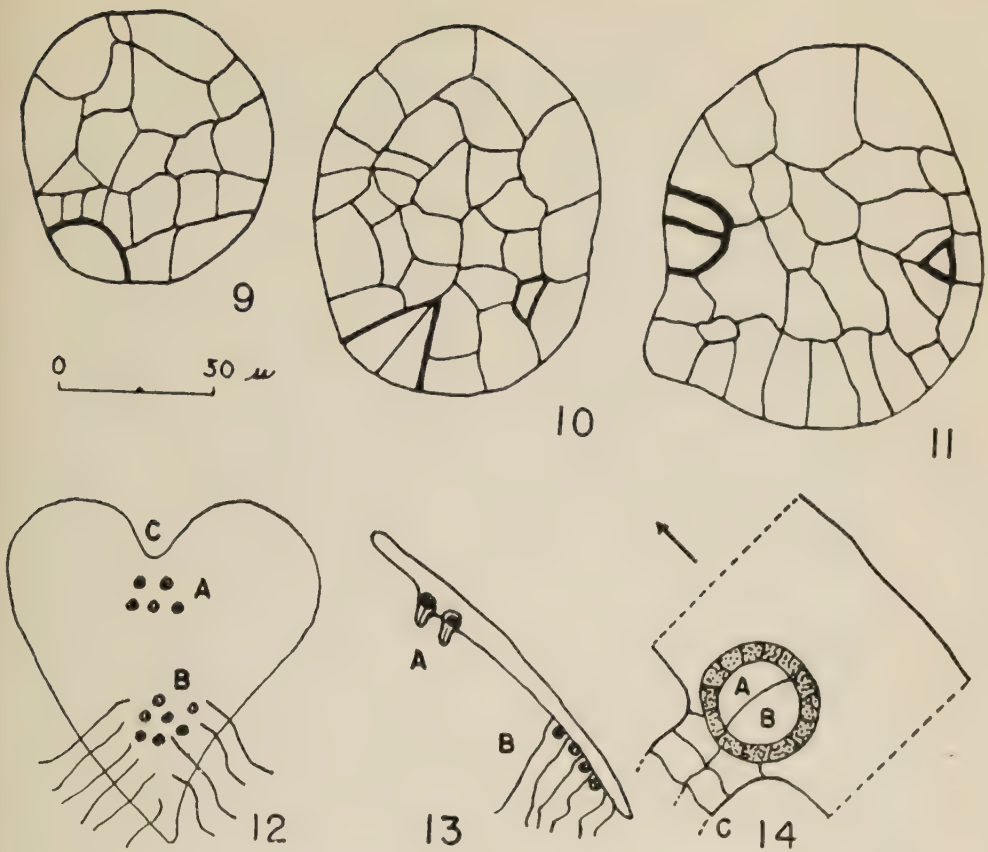
The mature egg in *Phlebodium aureum* rests in the venter of the archegonium and seems lightly adherent to the adjacent prothallial cells. A thin, membranous covering is evident in sectioned material, and the living cell yields readily to mechanical pressure when touched with a pointed object. A certain amount of granular content is emphasized in staining at this stage, and the egg shows signs of turgor. Immersion in a 15 per cent sucrose solution for ten minutes usually causes more or less withdrawal of the covering membrane from contact with some of the attendant venter cells. The exposed egg, at the time of fertilization and immediately after normal opening of the archegonium, is subject to plasmolysis by the usual fixing agents. This appearance is characteristically evident in the drawings by early workers in fern embryology.

The egg becomes less susceptible to extreme plasmolysis a few hours after fertilization, and the deep-staining contents are not present in the enlarging embryo until some time after the primary divisions. Neither the unfertilized nor the fertilized egg is stained deeply with Lugol's iodine. After fertilizaton, the egg shows more physical attachment to the prothallial cells, particularly in the region where the foot is likely to develop. The young zygote is then less readily removed from the parent cavity and reaction to plasmolytic treatments is less marked than before.

The fertilized egg in this species undergoes the first division rather uniformly in about five days. No first division walls were noted in any embryos in a shorter period of time. This division in all of several cases examined in detail is in a plane of the axis of the archegonium and at right angles to the anterior-posterior axis of the prothallium (Figs. 2, 14).

The second division in this species seems to follow the classically described pattern of being at right angles to the first wall and at right angles to the axis of the archegonium. A division then ensues immediately in each of the quadrants formed, producing the noted octants (units of the eight-celled stage), characteristic of the classical story for the higher ferns (just prior to the stage in Fig. 3). This takes place about six to eight days after fertilization. Soon after the octants are formed the identity of the four common derivative organs or appendages becomes clear. A review of the development of each structure follows in order of its appearance.

The Foot — This organ, classically noted for its early nutrition of the embryo and attachment of the young sporophyte to the prothallium, is also classically relegated to the end of most discussions on organ formation in the young embryo. In *Phlebodium aureum,* as studied presently, it is the first structure to be formed, often being evident in embryos eight to ten days old. Its early delimitation is reflected in the increase in size of its cells, and by the closeness of its contact with the prothallium. The organ is one of enlarged and vacuolated cells, a feature that remains distinguishing throughout its functional existence. A ten-day-old embryo, for instance, was noted to display a width in the median region of the foot of five greatly enlarged cells. An embryo, 20 days of age, has no more than twice as many cells, each quite enlarged, with some running to extreme dimensions of 40-50 microns. This size ranges up to three



FIGS. 9-14 — Figs. 9-11. Stages in the development of the embryo of *Phlebodium aureum*, showing initiation of primary appendages. Fig. 9. Heavily outlined cell in lower left likely gives rise to leaf at 10 days of age. Fig. 10. Leaf initials are outlined at left; cell outlined at right may give rise to root. Fig. 11. Cells outlined at left above leaf are stem initials, evident in 12-day-old embryo, and following root initiation. Figs. 12-14. Position of antheridia and archegonia on typical fern prothallium and the relation of the young embryo to the prothallium are shown diagrammatically. Fig. 12. Outline of prothallium, showing ventral surface, with apical notch at C. Archegonia and antheridia are at A and B, respectively. Rhizoids appear also at B. Fig. 13. Vertical longitudinal section of mature prothallium in general position of natural growth, showing archegonia at A and antheridia at B. Fig. 14. Five-day-old zygote in venter has divided into anterior ("epibasal") portion, A; and posterior ("hypobasal") portion, B. Apical notch is to the left (arrow). Remnant of archegonium neck is at C; cells of venter wall are shown stippled. Scale is applicable only to Figs. 9-11.

times that of other cells elsewhere in the embryo.

It is pertinent to the understanding of the foot to note from what part of the early embryo it is basically derived. As shown in Figs. 4, 5, 9, the foot must (at the eight- to ten-day stage) be regarded as including much of the area derived from the four upper octants and not solely from the upper posterior octants as often suggested (Eames, 1936, p. 273). This is of regular occurrence in *Phlebodium aureum*, and it may be regarded as a major disagreement with the rigid octant concept of appendage derivation. The enlargement of the foot cells is preceded by meristematic activity in the neighboring venter cells (Figs. 1-5). The later vacuolation and enlargement of these gametophyte cells parallel to that of the foot tissue cells. The walls of the foot cells in *Phlebodium aureum* are notably thin

throughout development, and there is no evidence of significant thickening.

For a time the cells of the adjacent calyptra give contrast to the appressed walls of the foot cells. This difference is less evident in the later stages of development, and the line of demarcation between the two areas is less abrupt. It is often difficult even at high magnifications to determine the boundary between the two tissues. This condition evidently results from a similar vacuolation and enlargement in the cells of the two regions. Meristematic activity in the calyptra ceases by the time the embryos are about 12-14 days old.

In sectioned embryos beyond the octant stage, there is no space between the foot and the calyptra cells in the area extending from the upper posterior half of the embryo to a region that includes most of the upper anterior portion (Figs. 3, 5). It is within this latter area that the stem classically develops.

While the calyptra cells are enlarging, they seem to yield to an increasing protrusion of the foot cells. The formerly symmetrical line of boundary between the tissues (Fig. 5) changes to an irregular one through the wedge-like extensions of the foot cells between the cells of the calyptra (Figs. 7, 8). In *Phlebodium aureum* this is comparable to the haustorium-like extension of the foot cells described widely in the classical accounts.

Both the foot and calyptra tissues are in close physical contact throughout development. No suggestion exists from the usual staining techniques of a space between the two for the accumulation of nutrients in any stage of transfer between the areas. The passage of products of nutrition must, therefore, proceed directly between the different cells.

At no time is there any evidence of physical or physiological breakdown of the prothallial cells in the region of the foot. The extension of the foot cells into the prothallium is only by intercellular intrusion. Embryos of almost any age may be separated clearly from the cells of the prothallium, precluding any likelihood that the tissues might have grown intimately together in the physical contact evident in all stages of development.

The First Leaf — The first leaf is initially evident in anticlinal divisions of surface cells derived uniformly from the area of the lower anterior octants in fair conformity with the classical story. Very early one cell or two assume a wedge shape in this surface area by the nature of the divisions. It is at this stage that it may properly be assumed that a single apical initial commands the growth of the new organ. In most cases the leaf initiation is clearly marked by the tenth or eleventh day after fertilization.

This single apical cell, if such it really is, later loses its clear identity in the widening and elongation of the tip of the organ. While this apparent condition of multiple initials is not always evident in the embryo prior to its breaking through the calyptra, it is present in the exposed and rapidly growing first leaf.

This organ is, therefore, characterized in *Phlebodium aureum* by: (1) a regular development from derivatives of the lower anterior octants, (2) exogenous origin by anticlinal divisions, (3) a tendency toward a single apical initial in the early stages, and (4) the supplanting of this initial by multiple cells in its development, particularly following rupture of the calyptra.

Procambial tissue develops acropetally in the enlarging leaf (Figs. 7, 8) from a median region of the embryo where the traces join those of the oppositely directed and endogenously developing root. The cells of this central area early assume a characteristic elongated form. Enlargement and vacuolation of the surface cells of the embryo below and posterior to the first leaf is evident in Figs. 7, 8. These form a tissue that portends the cortex in nature and position, though still not covered by a defined epidermis.

The First Root — The root in *Phlebodium aureum* originates endogenously in the general area of the derivatives of the lower posterior octants, just inside the superficial cells of the embryo (Figs. 6, 7, 10). The meristematic activity that first suggests such beginning is often quite near the assumed former boundary of the two sets of posterior octants. It seems inaccurate to assert that the organ is solely derived from lower posterior octant tissue. At the octant stage and immediately

afterward, there is no delimiting of cells that may be precisely ascribed to the impending root.

The inception of the root follows by about two days the divisions that mark the origin of the leaf. This differential in development is maintained with fair uniformity throughout the early development of the sporophyte. It is only at some time after the emergence of both organs through the calyptra that the root surpasses the leaf in rate of growth. The root initial or initials soon lose their identity in a region of meristematic activity.

Procambial activity follows the first divisions of the root by one or two days. The more or less elongate cells of procambium appear first in a median portion of the embryo and then make connection with the oppositely pointed leaf (Fig. 7). The orderliness of development of the procambium in the young root was not determined. Its appearance over the entire area from its connection with the leaf is so nearly simultaneous that a sequence of events is not readily observable. The root attains sufficient size to rupture the calyptra at a point opposite the leaf about a day after the emergence of the latter organ.

The Stem — This is the last of the primary organs to develop, and it originates in *Phlebodium aureum* from superficial cells derived from the original upper anterior octants. Yet, it is likely true that many of the derivatives of these same octants take part in the formation of the foot. It then follows that the much later development of the stem is from a region near the hypothetical border between the former upper and lower anterior octant regions. This is in an area dorsal to the first leaf and about midway between the extremities of the leaf apex and foot (Figs. 6, 11).

Stem formation is first suggested by anticlinal divisions in the outer cells of the embryo about two days after the initiation of the root (Fig. 11). The stem develops slowly and gains vascular connection by the advance of procambial strands upward at an angle from those already joining the leaf and root.

The exact disposition of the cells of the apical region was not considered in this study, nor was the origin of the second leaf followed.

The form of the young embryo of *Phlebodium aureum*, which now possesses the foot, first leaf, first root and stem initials, is that of an inverted T (Figs. 7, 8). In this representation, the leaf-root axis is the horizontal cross-bar and the vertical median axis of the foot intersects the middle region of the first line, forming the vertical bar of the inverted T. This general typification as to form, specifically usable for comparisons made in the morphogenetic studies reported separately, is generally reflected in essentially all the higher ferns. Atkinson (1894) and Goebel (1887) represented a comparable T-form in *Adiantum*. Campbell (1892) presented such a form in *Osmunda*; Goebel (1930) shows it as the leptosporangiate type.

Secondary thickening of the vascular cells is not yet in evidence at this stage (Figs. 7, 8), but the xylem areas are soon to be marked by heavy lignification readily susceptible to safranin stains.

Discussion

The classical accounts of fern embryology held that the foot originates in the upper posterior quadrant or the two derived octants (Leitgeb, 1878; Goebel, 1887; Campbell, 1892, 1905; Conard, 1908). Bower (1923) gave the origin of the foot as the " hypobasal hemisphere ".

Cross (1931), in a study of *Osmunda*, found that the foot was derived chiefly from the upper half of the embryo next to the gametophyte instead of from the upper posterior quadrant alone. Thompson and Hull (1934) survey with doubt the propriety of assigning initials of embryonic organs to parts as early as the quadrant stage.

The present study on *Phlebodium aureum* suggests, as previously outlined, that the origin of the foot is not restricted to one set of octants (upper posterior) but that its origin is in fact from much of the area certainly derived from both sets of upper octants. Not until later stages of development is the differentiation evident that clearly delimits the foot.

The steps in the development of the foot are generally agreed upon by the various workers in the field. Campbell (1892) notes that in *Osmunda* the cells do not possess any regular order of division, that the divisions are fewer and that the whole area enlarges. The boundary of the organ, he notes, is not clearly demarcated at any point from the rest of the embryo. At the time of rupture of the calyptra, the foot in *Osmunda* is roughly half of the embryo. He points out that this size is attained almost exclusively by simple expansion of the cells. Cross (1931) indicates also in *Osmunda* that here division is limited and that the cells undergo enlargement. The present study suggests that in *Phlebodium aureum* the foot develops as the first of the recognizable primary organs, that its divisions are few and slowly arrived at, and that the developmental pattern closely parallels that described for other species.

The relation of the cells of the foot to the neighboring cells of the prothallium is not clearly described in most reports on the embryology of ferns. It is classically recognized as an organ of nutrition, having close physical and physiological contact with the prothallium. Campbell (1892) held that the foot cells encroach upon the cells of the prothallium, penetrate deeply, and partially destroy the latter cells. His Figs. 95 and 96 do not show evidence of destruction of cells, and occasional wedge-like invasions of foot cells are less accented than may be noted often in *Phlebodium aureum* (Fig. 7). Conard records no details of the origin of the foot in his study of *Dennstaedtia*, although his figures show a general size difference in the cells of the foot and the remaining embryo. There is no evidence of marked insertion of foot cells between those of the prothallium.

Atkinson (1894) found the foot cells low in protoplasmic content, and ascribed it to an absorptive function and a temporary existence. His Fig. 48 shows the foot area in loose contact with the prothallial tissue. This is likely an effect of imperfect fixation, although Atkinson does not assign any significance to this condition. Conard (1908), Cross (1931), and Smith (1938) are among those who showed clearly the close contact of the two tissues. The closeness of this contact in *Phlebodium aureum* is thus in conformity with that represented in the more recent works in other species. At 14-16 days of age, as has been noted, it is often difficult to discern the boundary of the two adjoining tissues (Fig. 8). This condition of the prothallial cells is in sharp contrast to the very early stages of development in which the cells are smaller, meristematic, and receptive to deeper staining.

The origin and early development of the first leaf has been noted generally for *Phlebodium aureum*, and the findings are in general conformity with the classically described pattern. The appearance of the leaf as the structure formed just after the foot and before the root is likewise in harmony with the usually described leptosporangiate development.

There exists in the literature no real exception to the concept that the first leaf originates from the general aggregate of cells of the embryo derived from the original lower anterior octants. The origin of the leaf is ascribed directly to the definite quadrant (Goebel, 1887; Campbell, 1892; Eames, 1936) or to two octants (Conard, 1908), or to the anterior hemisphere, as assumed by Bower (1923). In *Phlebodium aureum* it may be said that, although no leaf is at all evident at the octant stage, the first leaf arises exogenously from the general area of the octant derivatives from two to four days after the octant stage was attained.

The inception of the leaf is marked by anticlinal divisions in the superficial area of the embryo, and a triangular cell or two at first is evident (Figs. 6, 9). The existence of a single apical cell for any considerable time was not confirmed, as that factor was not critically studied in *Phlebodium aureum*.

It is of interest that in *Dennstaedtia punctilobula*, Conard (1908) noted that the origin of the leaf from the two cells of the octants previously mentioned is never correlated with the appearance of a single apical cell. The two cells divide into a group of superficial initals that is thereafter maintained. Campbell (1892) describes a single apical cell that persists in

later development of the first leaf in *Osmunda*. Bower (1908) considered the tendency toward a single leaf initial an evolutionary advance in ferns.

The steps in the development of the first leaf from its inception until after it has extended through the calyptra are, by common agreement, essentially uniform in the higher ferns. *Phlebodium aureum* shows no exception. There is no evidence to suggest, however, that the young leaf is an absorptive organ while within the prothallial envelope, as was noted by Bower (1923) in referring to work of Goebel in the lower ferns which notably lack an extensive foot.

The leaf is green when it breaks through the calyptra in *Phlebodium aureum*, and it is consequently potentially capable of photosynthesis thereafter as it grows eventually upward through the apical notch.

Bower (1923) regards the root in the leptosporangiate ferns as always originating in the posterior half of the embryo. Campbell in *Osmunda* (1892) shows the endogenous root initial in an almost median position of the posterior half of the multicellular embryo. Conard (1908) illustrates the early divisions of the root as being definitely in the multicellular mass of cells derived from lower posterior octants (his Fig. 236). He is clear in stating that the root may originate basically from either one of the octants mentioned. Goebel (1887) also found the origin of the root to be from either of the usual octants.

The region of root origin is apparently one of uniformity in the various species of higher ferns. The endogenous beginning is generally agreed upon, although Campbell (1892, Fig. 91) assigns to a superficial cell the role of root cap obviously long before further divisions could properly delimit tissue seen to be a root.

In *Phlebodium aureum* there is no clear reason for assuming the presence of a single apical cell in the development of the root, although this aspect of development was not considered in these studies. Campbell (1892) found the presence of a single apical cell of uniform occurrence, even while he refers to Bower's contention that a group of initials may be present in *Osmunda*.

The development of the root initials following the definition of the first leaf in *Phlebodium aureum* is in agreement with the classical picture. It is likewise true that, although the root follows the first leaf in rupture of the calyptra, it soon exceeds the leaf in rate of growth.

It is almost sheer presumption to attribute the origin of the stem in *Phlebodium aureum* to a quadrant, as did Campbell (1892) or to one of a pair of octants as did Goebel (1887) and Conard (1908) in other species. Yet the general region of origin in respect to the entire embryo follows the same pattern. It assumes a position of secondary prominence in regard to the oppositely pointed and horizontally directed leaf and root. It is uniformly the last of the primary organs to develop, and its origin usually may be recognized a day or two before the first leaf and root emerge from the covering prothallial tissue. At this stage, procambial tissue formation is already under way, linking with the established leaf-root axis of procambial cells. Later development of the stem was not followed in these studies.

The weight of evidence, as drawn from the study of *Phlebodium aureum*, indicates clearly that too much stress has been given to the quadrant and octant concepts in fern embryology. Any significance to be attached to the areas seems at best to be only topographic. It is quite possible that plant embryology has been made more difficult by the attempt to force farther and farther back the origin of organs to specific cells.

Summary

This paper reviews the development of the embryo of *Phlebodium aureum* through the stages of formation of the foot, first leaf, root, and stem. The time required for such development is found to be about 14 days, and the emergence of the young embryo through the gametophyte tissue follows in another day or two.

In this species the first division of the embryo occurs about the fifth day after fertilization, after progressive enlargement of the single cell. Subsequent divisions occur rapidly; the massive stage

beyond the octant divisions is apparent by the eighth day; and almost immediately thereafter the primary structures show signs of origin, being set apart by about a day in order of their appearance.

Growth of the foot results in an interlocking of the bordering foot-prothallial cells, but there is no evidence of destruction of the prothallial tissue. This latter tissue becomes vacuolate in its later stages and resembles in structure and content the cells of the foot.

The origin of these first appendages cannot with exactness be assigned to a quadrant or to either of the later octants. Recognizable delimitation of the parts follows after many cell divisions and much consequent growth. The organs do, however, appear in the regions of derivatives of the original octants. This origin is clear in the case of the foot and leaf, and less so in the case of the root and subsequent stem.

The foot is seen to include much of the derivative tissue of the upper anterior octants as well as the classically described upper posterior octants. The first leaf has its incidence in the general region of the derivative cells of the lower anterior octants. The origin of the stem is not clearly within the region of upper anterior octants but from a region that may properly be regarded as between the two sets of anterior octants. The root arises from the general area of the former lower posterior octants.

The studies reported herein were carried on chiefly at the Biological Laboratories, Harvard University. The extended assistance of Prof. R. H. Wetmore throughout the studies is gratefully acknowledged. A part of the work was facilitated by equipment provided by the Bache Fund of the National Academy of Sciences. It is a distinct pleasure to acknowledge this assistance.

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LEAF NITROGEN IN RELATION TO STRUCTURE OF LEAVES OF PLANTS GROWING IN GYPSUM SAND[1]

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Introduction

For a given species leaf nitrogen is known to vary with soil fertilizer treatment (Beeson, 1941), leaf age (Pochon & Lajudie, 1948), and, to some extent, with time of day (Miller, 1938). In the present study nitrogen determinations made on mature, functional leaves of a number of plant species growing in gypsum sand are considered in relation to leaf structure and available soil nitrogen. Micro-Kjeldahl analyses for total and for amino nitrogen were made on leaves of forty and on stems of two of the more than seventy plant species supported chiefly by the marginal dunes and intervening flats of this drifting gypsum substrate which is twenty-eight miles in length and eight to ten miles wide. Structurally the leaves exhibit various combinations of mesomorphic, succulent and/or xeromorphic characteristics. The sand, which is 97 per cent calcium sulfate, is low in phosphorus as well as nitrogen and possibly is low in potassium also. While the moisture content of the dunes is negligible except immediately following precipitation, the water table in the flats lies within two to three feet of the sand surface.

Methods and Materials

Ten to twenty-gram samples of mature leaves from the middle stem region were collected on the mornings of May 15 to 17 and August 8 to 10. The leaves were weighed at the collection site, dehydrated in a laboratory oven at a temperature of 80°C. and again weighed for estimating moisture content. Samples were pulverized and brought to a constant weight for determining total nitrogen by a modification of the micro-Kjeldahl method to include nitrites and nitrates (Loomis & Shull, 1937). The modification involved the addition of salicylic acid to the digestion mixture to bind nitrate in organic combination. Sodium thiosulfate was added after thirty minutes to reduce the nitrite group with the formation of amino salicylic acid. By the Kjeldahl method organic nitrogen is converted to ammonium sulfate, followed by the liberation and acidimetric titration of ammonia. When analytical results for leaves of a particular species in one collection series differed by more than 0·5 per cent, both were rejected and a duplicate analysis was repeated. Blank determinations were run on a cigarette paper of the same kind as the one containing the micro-sample, using the same amounts of reagents without the plant material. Values of blank samples were subtracted from the results of each analysis. The percentage of total Kjeldahl nitrogen for leaves of each species was determined in duplicate in two to four separate series. Amino nitrogen only was determined by micro-Kjeldahl analysis (Niederl & Niederl, 1947) of duplicate samples of one leaf series collected in August.

Total soil nitrite and nitrate were determined for samples taken from a depth of from six to eighteen inches below the surface at the base of each plant for which leaf nitrogen analyses were made in May[2].

2. Grateful acknowledgement is made to Dr. J. L. Gardner and M. L. Seal, College Station, New Mexico, for the soil analyses.

1. This study was subsidized by a grant from the National Science Foundation

Transverse sections of typical mesomorphic, succulent and xeromorphic leaves were illustrated by projecting the tissue directly to photographic enlarging paper, tracing the image with India ink and bleaching the photographic impression.

Observations

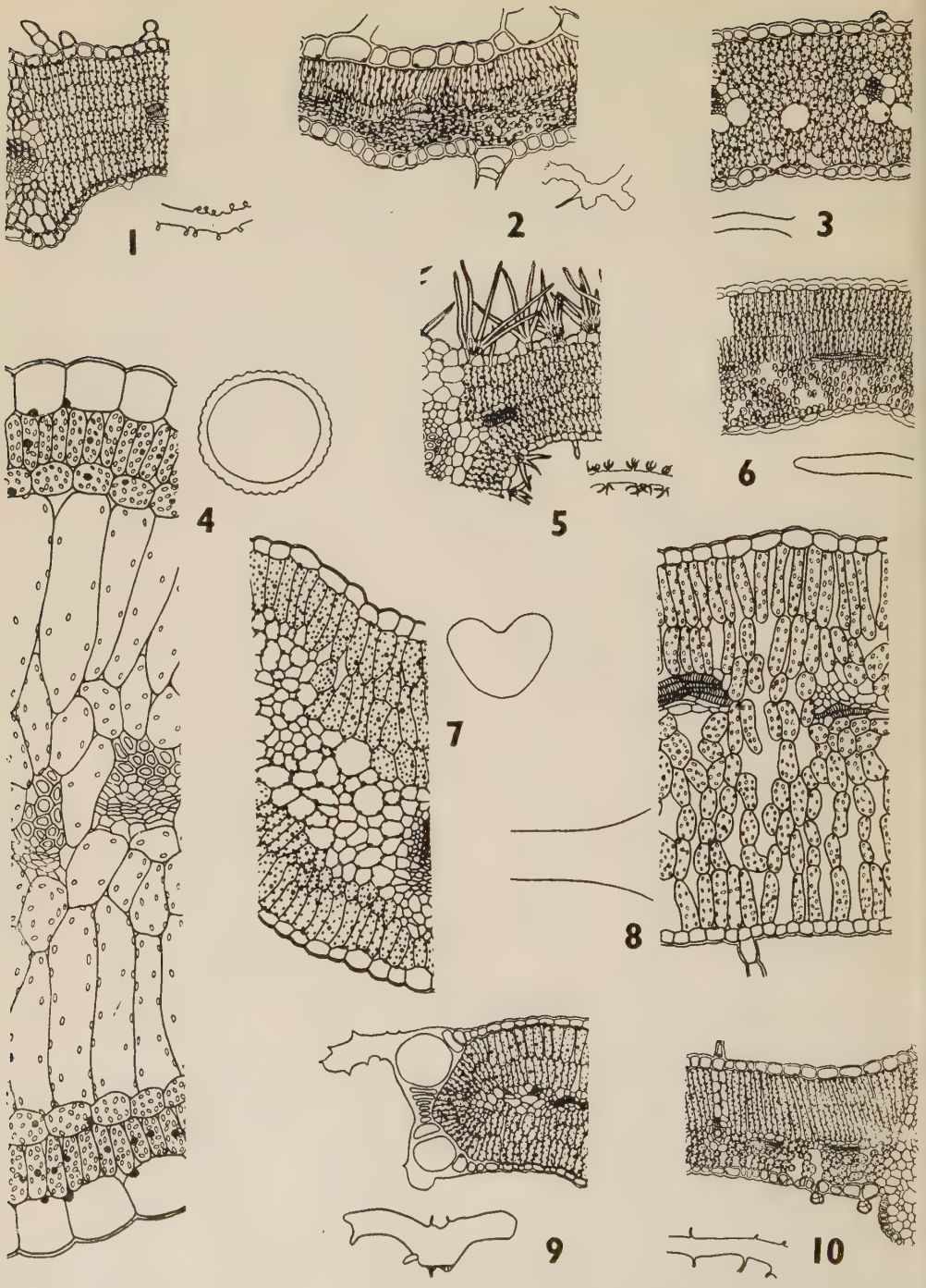
LEAF NITROGEN — Table 1 lists the species for which leaf analyses were made, arranged in order of decreasing total micro-Kjeldahl nitrogen concentration in

TABLE 1 — TOTAL AND AMINO MICRO-KJELDAHL NITROGEN OF MATURE LEAVES FROM THE MIDDLE STEM REGION EXPRESSED AS A PERCENTAGE OF DRY WEIGHT; SOIL NITRATE PLUS NITRITE AND SOIL PHOSPHATE FROM THE VICINITY OF ABSORBING ROOTS OF EACH PLANT FOR WHICH TOTAL LEAF NITROGEN WAS DETERMINED; AND MOISTURE CONTENT OF LEAVES COLLECTED IN AUGUST FOR MICRO-KJELDAHL ANALYSIS

Species	May			August		
	Total leaf nitrogen	Total soil NO_2 and NO_3 (p.p.m.)	Soil PO_4 (p.p.m.)	Total leaf nitrogen	Amino nitrogen	Moisture (per cent of fresh wt.)
1. *Helianthus annuus* L.	5·77	2·5	4·0	5·66	3·98	74·0
2. *Cucurbita foetidissima* H.B.K.	5·05	1·0	3·5	4·35	3·37	83·5
3. *Aster cichoriaceus* (Greene) Blake	5·02	4·5	3·5	4·39	3·36	71·1
4. *Suaeda suffrutescens* S. Wats.	4·90	1·0	2·0	4·40	3·15	82·0
5. *Sphaeralcea incana* Torr.	4·48	1·5	5·0	4·33	3·77	63·0
6. *Prosopis juliflora* var. *glandulosa* (Torr.) Cockerell	4·15	1·0	6·0	3·90	3·00	69·1
7. *Thelesperma megapotamicum* (Spreng.) Kuntze	3·85	2·0	2·5	3·50	2·89	80·7
8. *Asclepias arenaria* Torr.	3·57	2·5	3·5	3·20	2·85	79·0
9. *Mentzelia integra* H.B.K.	3·53	2·0	5·0	3·25	2·62	76·7
10. *Rhus trilobata* (Nutt.) ex Torr. and Gray	3·50	1·0	2·5	3·48	1·90	56·5
11. *Haplopappus spinulosus* (Pursh) D.C.	3·30	3·0	4·0	3·20	2·95	58·7
12. *Chrysothamnus nauseosus* (Pall.) Britton var. *latisquameus* (A. Gray) H. M. Hall	3·17	5·0	4·0	3·10	2·40	60·0
13. *Haplopappus heterophyllus* (A. Gray) Blake	3·09	3·0	7·5	3·38	—	76·2
14. *Abronia angustifolia* Greene	3·05	3·0	2·5	2·81	2·58	81·2
15. *Greggia camporum linearifolia* Jones	3·01	1·0	2·5	2·70	2·20	84·2
16. *Coldenia hispidissima* (Torr.) A. Gray	2·98	1·0	3·5	2·85	2·80	68·5
17. *Chrysothamnus pulchellus* (Gray) Greene	2·85	1·5	2·0	2·71	2·05	54·5
18. *Clappia suaedifolia* A. Gray	2·77	1·0	3·5	2·80	2·32	82·0

TABLE 1 — TOTAL AND AMINO MICRO-KJELDAHL NITROGEN OF MATURE LEAVES FROM THE MIDDLE STEM REGION EXPRESSED AS A PERCENTAGE OF DRY WEIGHT; SOIL NITRATE PLUS NITRITE AND SOIL PHOSPHATE FROM THE VICINITY OF ABSORBING ROOTS OF EACH PLANT FOR WHICH TOTAL LEAF NITROGEN WAS DETERMINED; AND MOISTURE CONTENT OF LEAVES COLLECTED IN AUGUST FOR MICRO-KJELDAHL ANALYSIS — *Contd.*

Species	May			August		
	Total leaf nitrogen	Total soil NO_2 and NO_3 (p.p.m.)	Soil PO_4 (p.p.m.)	Total leaf nitrogen	Amino nitrogen	Moisture (per cent of fresh wt.)
19. *Andropus carnosus* (Wooton) Brand	2·75	0·5	2·5	2·85	2·09	84·0
20. *Senecio spartioides* Torr. and Gray	2·70	2·5	3·0	—	2·05	70·0
21. *Gutierrezia sarothrae* (Pursh) Britt. and Rusby	2·70	2·0	5·5	2·75	1·81	65·7
22. *Lycium torreyi* A. Gray	2·69	2·5	4·0	2·70	2·32	68·5
23. *Baccharis glutinosa* Pers.	2·65	1·0	3·5	2·68	1·39	63·0
24. *Populus wislizeni* (Wats.) Sarg.	2·60	1·0	3·0	2·46	1·77	60·0
25. *Psilostrophe tagetinae* (Nutt.) Greene	2·52	2·0	3·5	2·47	2·39	80·5
26. *Oenothera filifolia* (Eastw.) Tidestrom	2·44	1·5	2·5	2·40	1·97	80·0
27. *Poliomintha incana* (Torr.) A. Gray	2·40	4·0	2·5	2·15	1·76	64·7
28. *Atriplex canescens* Pursh	2·37	1·0	2·5	2·22	1·63	36·5
29. *Frankenia jamesii* Torr.	2·34	1·5	3·5	2·30	1·58	62·0
30. *Hymenopappus arenosus* Heller	2·07	1·0	2·5	1·64	1·58	61·5
31. *Comandra pallida* A.D.C.	1·89	2·5	3·0	1·77	1·40	70·1
32. *Ephedra torreyana* S. Wats.	1·87 (stem)	3·0	2·5	1·90 (stem)	1·88	70·2
33. *Lycium berlandieri* Dunal var. *parviflorum* (Gray) Terrac.	1·80	2·5	4·0	1·86	1·79	60·1
34. *Sporobolus airoides* Torr.	1·75	3·5	2·0	1·85	1·77	56·9
35. *Oryzopsis hymenoides* (Roem. and Schult.) Ricker	1·65	4·5	2·5	1·56	1·35	49·0
36. *Muhlenbergia pungens* (Thurb.) Rydb.	1·58	2·5	4·5	1·63	1·22	50·2
37. *Sporobolus giganteus* Nash	1·39	2·5	3·0	1·43	1·33	52·4
38. *Yucca elata* Engelm.	1·27	3·0	2·5	—	—	64·5
39. *Bouteloua breviseta* Vasey	1·25	—	—	1·50	1·45	56·0
40. *Andropogon scoparius* Michx.	1·23	2·5	3·0	1·36	1·36	55·8
41. *Distichlis spicata* (L.) Greene	1·20	0·5	7·0	1·26	1·14	50·2
42. *Juncus mexicanus* Willd.	1·00 (stem)	1·5	4·0	1·15 (stem)	1·07	62·2



FIGS. 1-10 — Figs. 1-3, 5, 6, 9, 10. Mesomorphic leaves, in transverse section, numbered in order of diminishing total nitrogen concentration in May, ranging from 5·77, high, to 3·50 per cent of the dry weight. Fig. 1. *Helianthus annuus* L., Fig. 2. *Cucurbita foetidissima* H.B.K., Fig. 3. *Aster cichoriaceus* (Greene) Blake, Fig. 5. *Sphaeralcea incana* Torr., Fig. 6. *Prosopis juliflora* var. *glandulosa* (Torr.) Cockerell, Fig. 9. *Mentzelia integra* H.B.K. and Fig. 10. *Rhus trilobata* (Nutt.) ex Torr. and Gray. Figs. 4, 7, 8. Three succulent leaf types in transverse section, total nitrogen in May ranging from 4·90, high, to 3·57 per cent of the dry weight. Fig. 4. *Suaeda suffrutescesu* S. Wats., Fig. 7. *Thelesperma megapotamicum* (Spreng.) Kuntze and (8) *Asclepias arenaria* Torr.

May. Illustrations of transverse sections of representative leaves are also shown in order of diminishing nitrogen content in this same collection series (Figs. 1-27). As the slight difference in the leaf nitrogen of species adjacent or close together in Table I suggests, this order of arrangement is not fixed and inflexible. The position of several species is somewhat different when listed in order of diminishing total nitrogen concentration in August, and still more differences appear when species are arranged according to decreasing amino nitrogen.

Without exception, however, both total and amino nitrogen are highest in all series in certain mesomorphic leaves of herbaceous species, total nitrogen of this leaf type (Figs. 1-3, 5, 6, 9, 10) ranging in May from 3·50 in *Rhus trilobata* to 5·77 per cent of the dry weight in *Helianthus annuus*. Leaves in this group are spatulate to broadly triangular in entire outline with a thin mesophyll composed of compact but short palisade cells in four species (Figs. 1, 3, 5, 9) and in three species of a typical palisade and spongy mesophyll (Figs. 2, 6, 10). These leaves at maturity measure 133 to 272 μ in thickness in central areas removed from major veins. *Helianthus annuus*, heading the list with an observed range in total nitrogen of from 5·66 (August) to 5·77 (May) per cent of the dry weight, had the highest concentration of total and amino nitrogen in all series.

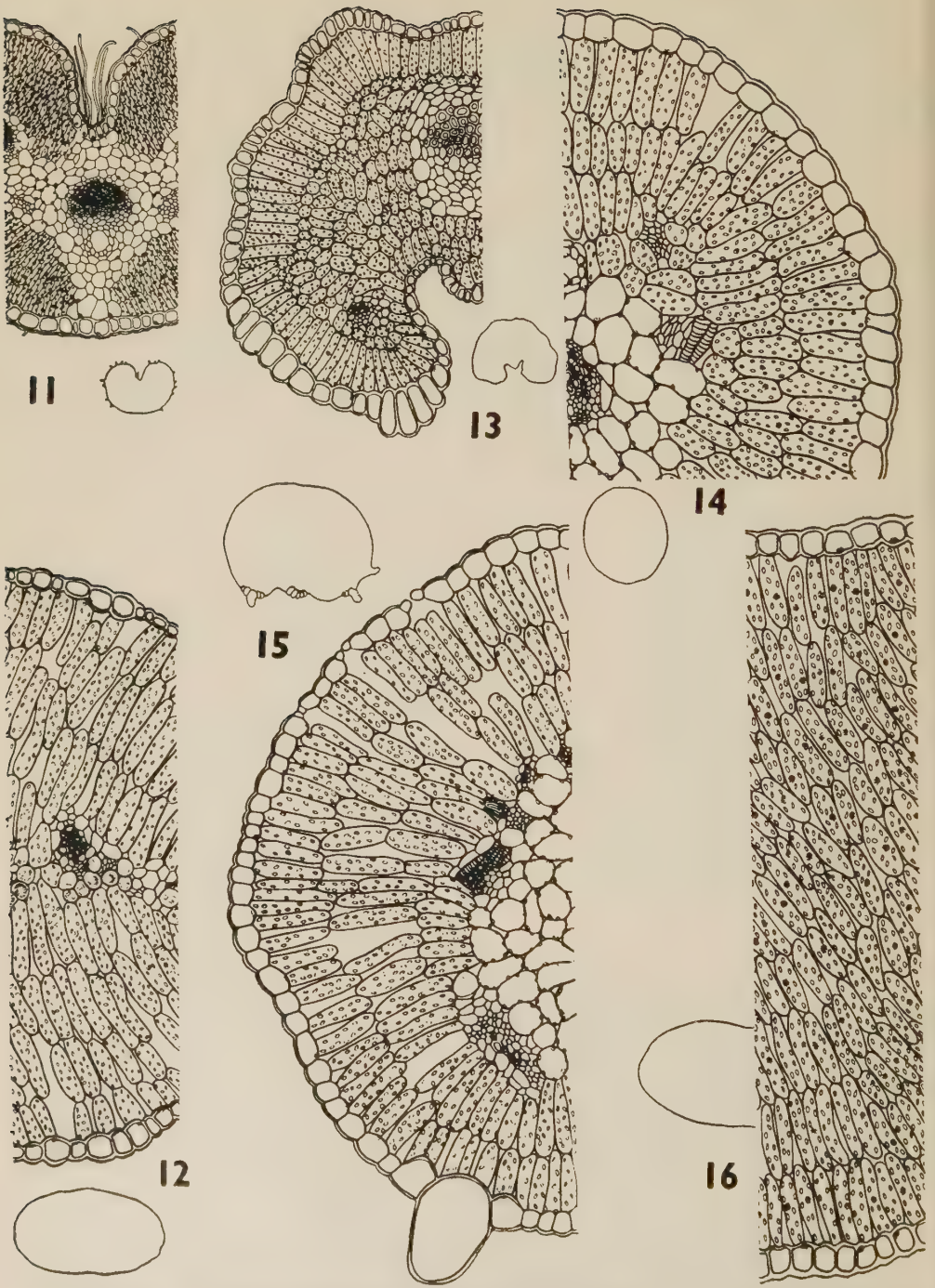
Similarly, in all series the succulent leaf type (Figs. 4, 7, 8, 11-16) constitutes the group second high in nitrogen concentration, total nitrogen in May ranging from 2·70 per cent of the dry weight in *Senecio spartioides* through 3·30 per cent in *Haplopappus spinulosus* (not illustrated) to 4·90 per cent in *Suaeda suffrutescens*, one of the group of three succulents which overlaps the mesomorphic leaved species in nitrogen content. These succulent leaves, which are linear-terete in entire outline with multiple upper and lower palisade layers numbering up to ten, are thickened by the dorsiventral development of water storage tissue, and vary in thickness from 359 μ in *Haplopappus spinulosus* to 1,602 μ in *Senecio spartioides* and 1,958 μ in *Suaeda suffrutescens*.

The mesomorphic leaves of the woody species *Lycium torreyi*, *Baccharis glutinosa* and *Populus wislizeni*, varying from 239 μ in thickness in *Baccharis glutinosa* to 432 μ in *Lycium torreyi*, appear in the lower half of Table 1 with a total nitrogen content of around 2·68 per cent of the dry weight, which is relatively low for a leaf with a broad, thin blade. Total nitrogen of the xeromorphic leaves of the shrubby *Poliomintha incana* and *Atriplex canescens* is approximately 2·40 per cent of the dry weight. Two non-shrubby species of this low nitrogen group, *Frankenia jamesii* and *Hymenopappus arenosus*, have the inrolled margins and reduced external surface characteristic of many xeromorphic leaves, but are relatively thin, measuring 133 and 266 μ respectively in thickness.

Total leaf nitrogen was in most cases higher in May than in August, the increase in cell wall material, vascular elements, and other inert components as the leaf grows older diluting the nitrogenous constituents. Total leaf nitrogen in *Helianthus annuus* exceeds amino nitrogen of leaves in the same series by 1·78 per cent, the widest gap observed between total and amino nitrogen in comparable samples collected on the same date. Other experimental work has shown that concentrations of 50 mg. nitrate per gram dry weight, or 5 per cent, are frequent in leaves and roots where the rate of nitrate uptake exceeds the rate of nitrate reduction (Bonner, 1950). In the present study the concentration of amino nitrogen most nearly approaches total nitrogen in leaves which are low in total nitrogen content.

Most of the leaves lowest in nitrogen, 20-42 in Table 1, excepting 32 and 42, which are leafless stems, are xeromorphic, usually narrowly linear, dicotyledon leaves several of which are densely covered with trichomes; *Yucca elata* leaf, and grass leaves which are composed largely of mechanical tissue and non-green cells containing water. The two leafless stems, *Ephedra torreyana* and *Juncus mexicanus*, numbers 32 and 42 respectively in Table 1, are also low in nitrogen.

For purposes of comparison total leaf nitrogen was determined for samples from three of these species growing on range



FIGS. 11-16 — Transverse sections of succulent leaves thickened by a dorsiventral development of enlarged palisade cells as well as water storage tissue, ranging in total nitrogen in May from 3·17, high, to 2·70 per cent of the dry weight. Fig. 11. *Chrysothamnus nauseosus* (Pall.) Britton var. *latisquameus* (A. Gray) H. M. Hall. Fig. 12. *Greggia camnorum linearifolia* Jones. Fig. 13. *Coldenia hispidissima* (Torr.) A. Gray. Fig. 14. *Clappia suaedifolia* A. Gray. Fig. 15. *Andropus carnosus* (Wooton) Brand. Fig. 16. *Senecio spartioides* Torr. and Gray.

land where the total nitrite and nitrate amounted to 15 p.p.m. in May. The total nitrogen was found to be 5·94 per cent for young and 5·39 per cent of the dry weight for older leaves of *Cucurbita foetidissima*, 3·15 for *Rhus trilobata* and 2·85 for leaves of *Atriplex canescens*, compared to 5·05 per cent total nitrogen for leaves of *Cucurbita foetidissima*, 3·50 for *Rhus trilobata* and 2·37 per cent for *Atriplex canescens* growing in the gypsum substrate.

Soil Nitrogen—Detectable nitrite and nitrate in the gypsum substrate appear to bear no relation to either leaf nitrogen or the location at which the sample is collected with respect to marginal areas or flats and dunes farther in the interior of the gypsum deposit. The plants bearing the leaves in second and fourth places for total leaf nitrogen grow in a substrate in the second to the lowest position for nitrogen, with a total concentration of nitrite and nitrate of 1 p.p.m. A localized spotting in nitrogen distribution is characteristic of all parts of the gypsum substrate, but no consistent differences are apparent in the nitrogen concentration of the dunes and the interdunal depressions.

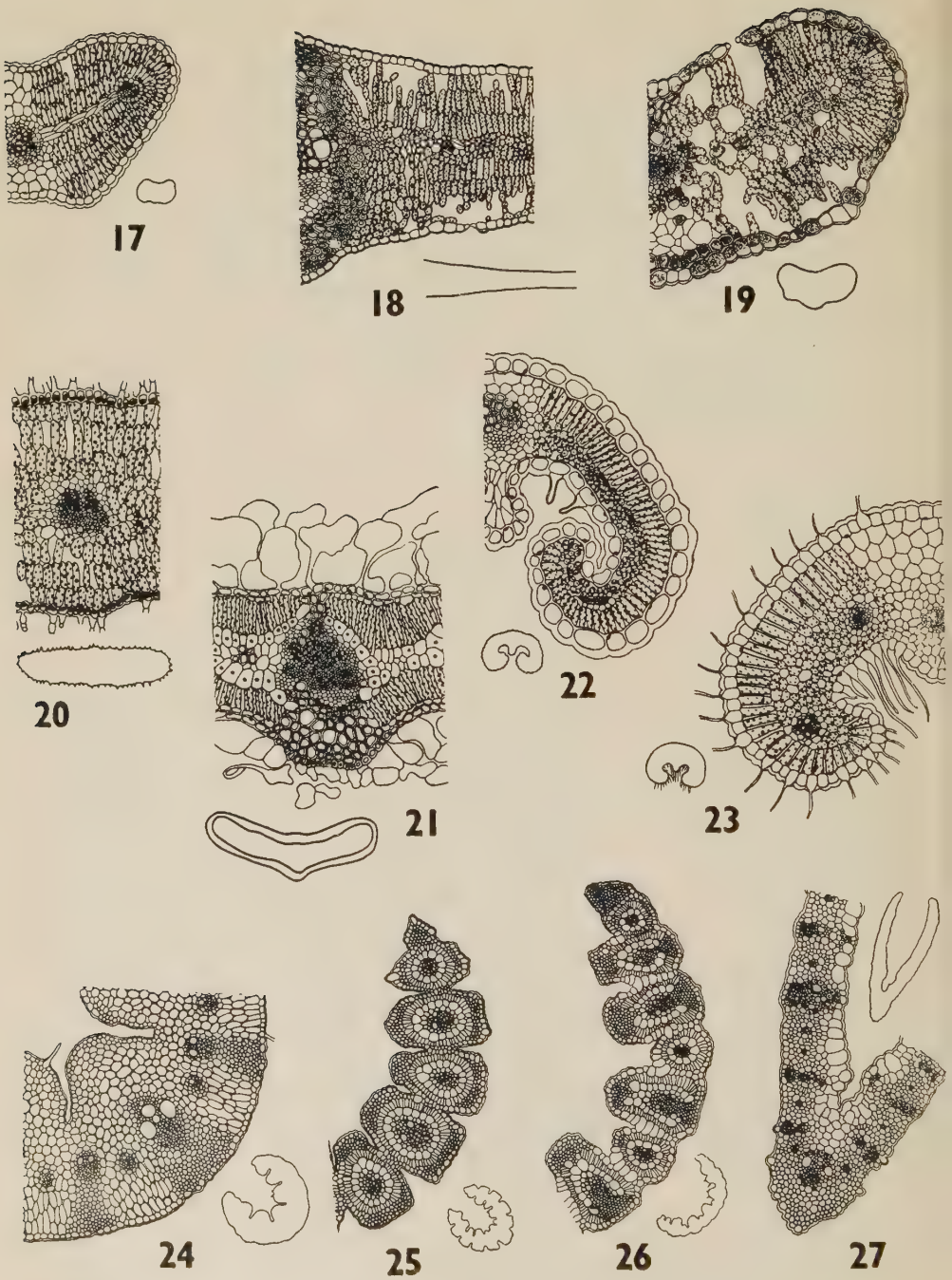
A number of gypsum samples, particularly those from peripheral flats, are capable of nitrification in ammonium sulphate solution over a period of three months, indicating the presence of a nitrifying microflora, however small, possibly incidental and resulting from recent wind-blown contamination. It would appear that soluble nitrogen compounds are produced sporadically throughout the gypsum deposit, but are almost immediately utilized, leached or lost by volatilization. The negligible carbohydrate content of the gypsum sand is one factor limiting the development of a vigorous saprophytic nitrogen fixing flora.

Phosphate concentration was determined for each sand sample, but no attempt is made in this study to note interactions resulting from low nitrogen and low phosphorus. All samples of the substrate showed over 500 p.p.m. potassium, but this figure is considered unreliable because of interference by the calcium ion.

Leaf Moisture—In general, an abundance of nitrogen increases the water supply of plant tissues (Miller, 1938). Because of the water within xylem elements in heavily vascularized leaves and the hydration of cell walls, however, moisture percentage is not necessarily related to nitrogen concentration. While the average moisture content in August is 72·7 per cent of the fresh weight for the twenty leaves highest in nitrogen, 1-20 in Table 1, and 60·2 for the twenty leaves with the lowest nitrogen concentration, the moisture content is 60 per cent or below in four of the first twenty leaves and above 72 per cent in two of the last twenty. *Atriplex canescens*, which has the lowest moisture content, 36·5 per cent of the fresh weight, is in the thirteenth position above the plant lowest in nitrogen in May. *Atriplex* spp. apparently have a high osmotic concentration, being especially tolerant of high soil salt. The leaves listed in Table 1 are not strictly comparable in moisture content since collections were made during the morning hours of three different days. Also, since sand in the dunes is dry except immediately following precipitation and

TABLE 2 — CONCENTRATION OF PROTEIN IN SUCCESSIVE LEAVES OF BARLEY PLANTS GROWN ON HIGH AND LOW NITROGEN SUBSTRATES (FROM GREGORY)

Leaf	Protein nitrogen in per cent of dry leaf		
	Full nitrogen substrate	1/9 of full nitrogen substrate	1/81 of full nitrogen substrate
3th leaf from base (youngest analysable)	3·23	3·12	1·81
3rd leaf from base	2·58	1·06	1·06



FIGS. 17-27.

the water table lies within two to three feet of the surface in the interdunal depressions, the moisture content is influenced by plant location with respect to the dunes or flats and by the depth of penetration of the root system, factors which cause no apparent variation in leaf nitrogen. Environmental moisture conditions may indirectly affect leaf nitrogen as where under xeric conditions the nitrogen absorbed is immediately used in protein synthesis in the root. Leaf moisture content in May was in all cases within 6 per cent of that in August. Leaves of woody species were lower in moisture, as well as nitrogen, than structurally similar leaves of herbaceous species.

Discussion

Each method of nitrogen determination has certain limitations. As plant material is dried for Kjeldahl analysis, some nitrogen is lost, and there is an increase in total water-soluble nitrogen, including ammonia, which may volatilize (Chibnall, 1922). Because of the considerable distance between the collection site and the laboratory, however, the Kjeldahl method was considered the most practical for this study.

Leaf Nitrogen — More than half the leaves used for analyses exhibit various combinations of a number of characteristics identified with xeromorphy, including reduced external surface and increased thickness associated with the development of multiple palisade layers at the expense of spongy mesophyll; thick cell walls, extensive vascularization, a heavy cuticle and a dense covering of trichomes (Shields, 1951). The dicotyledon leaves lowest in Kjeldahl nitrogen are those which are heavily vascularized, have small mesophyll cells, and, as evidenced by the diagrammatic cross-sectional outlines below each drawing in Plate III in contrast to those in Plates I and II, expose a much reduced external surface.

Leaf nitrogen should be proportional in some degree to the relative leaf volume occupied by living protoplasts. Nitrogen is comparatively low in leaves characterized by a dense covering of trichomes, a thick cuticle or low chlorophyll concentration and in those in which there is a high proportion of vascular tissue, sclerenchyma or cell wall material as where cells are small or thick walled. Leaves of high protein content are also high in chlorophyll (Bonner, 1950). Since leaves of xerophytes have a decreased total chloroplast surface (Priestley, 1929; Thoday, 1931) and possibly paler chloroplasts as where there is excess soil salt (Weaver & Clements, 1929), a lower nitrogen concentration would be associated with these characteristics as well as with the greater amounts of cell wall material, cuticle, trichomes and supporting tissue in this leaf type.

Leaves which are xeromorphic in appearance as to reduced external surface, increased thickness and multiple palisade layers, however, are often composed of cells which are large, thin-walled and well-stocked with protoplasm, resembling strict succulents in cell size and hydration. Since greatly hydrated living cell substance is high in comparison to dry weight, these leaves are high in nitrogen. In certain plants a xeromorphic-succulent type of leaf, having both conspicuous

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Figs. 17-27 — Figs. 17, 19-23. Transverse sections of representative xeromorphic dicotyledon leaves numbered in order of diminishing total nitrogen content in May, ranging from 2·70 per cent, high, to 2·07 per cent of the dry weight. Fig. 17. *Gutierrezia sarothrae* (Pursh) Britt. and Rusby. Fig. 19. *Oenothera filifolia* (Eastw.) Tidestrom. Fig. 20. *Poliomintha incana* (Torr.) A. Gray. Fig. 21. *Atriplex canescens* Pursh. Fig. 22. *Frankenia jamesii* Torr. Fig. 23. *Hymenopappus arenosus* Heller. Fig. 18. Transverse section from the leaf of *Populus wislizeni* (Nutt.) Greene, a broad, thin blade of a woody species which is lower than comparable leaves of herbaceous species in total nitrogen, amounting in May to 2·60 per cent of the dry weight. Figs. 24-27. Transverse sections of grass leaves characterized by a high proportion of mechanical tissue and ranging in total nitrogen in May from 1·75, high, to 1·20 per cent of the dry weight. Fig. 24. *Oryzopsis hymenoides* (Roem. and Schult.) Ricker. Fig. 25. *Muhlenbergia pungens* (Thurb.) Rydb. Fig. 26. *Bouteloua breviseta* Vasey. Fig. 27. *Andropogon scoparius* Michx.

palisade and water storage tissue as in *Andropus carnosus*, is produced by high salt and low nitrogen (Mothes, 1932), the situation in the gypsum sand. Succulence is associated with the conversion of polysaccharides into pentosans or mucilages, and pentosans in combination with nitrogenous substances have an enormous hydration capacity (MacDougal, Richards & Spoehr, 1919). Greatly hydrated nitrogenous components of the protoplasm are in part responsible for succulence. The relatively high nitrogen content of succulent leaves is related to the large, greatly hydrated protoplasts and a proportionally small amount of cell wall material and mechanical tissue.

The total nitrogen concentration in May of 1·20 to 1·75 per cent in the leaves of the seven grass species analysed in this study is relatively low. Nitrogen of the fourth leaf of nitrogen deficient barley plants has been caused to drop from 1·82 to 0·19 per cent of the dry weight over a period of twenty days, however, yet these leaves remained entirely capable of protein synthesis when nitrogen was supplied (Walkley, 1940). The average amino leaf nitrogen for a number of crop plants at different periods of the day, expressed as a percentage of dry weight, has been found to be 3·2 to 3·8 for corn, 3·6 to 4·2 for kafir, 4·5 for pumpkin, 4·5 for garden bean, 5·6 for cowpea and 5·8 for soybeans (Miller, 1938), the monocotyledon leaves, having a higher proportion of mechanical tissue, being lowest in this study also. The amino nitrogen content of potato leaves on a dry weight basis has been observed to range from 2·75 to 3·25 per cent in untreated soils to 4·50 to 5·25 per cent in soils given different fertilizer treatments, including various combinations of nitrate, phosphorus and potassium (Beeson, 1941). The protein nitrogen in barley leaves varies from 3·23 per cent in the youngest leaves to 4·06 per cent in mature and 2·52 per cent in the oldest leaves (Pochon & Lajudie, 1948). The decreased concentrations in the older leaves may result in part from the dilution of protein by increasing amounts of cellulose and other inert materials and to withdrawal of nitrogen to more active growth centers (Bonner, 1950).

Leaves of woody species have a higher cell sap concentration than leaves of annuals (Korstian, 1924). This higher osmotic pressure, a distinguishing feature of increasing xerophytism, is associated in the present study with a lower nitrogen content in the mesomorphic leaves of the woody species *Rhus trilobata*, *Populus wislizeni*, *Lycium torreyi* and *Baccharis glutinosa* than in structurally similar leaves of herbaceous species. The low osmotic pressure of succulents, on the other hand, results from excessive hydration and has little significance in water conservation in this group.

Soil Nitrogen — While the species highest in leaf nitrogen do not appear in the inner dunes or intervening flats, it is the drifting substrate rather than variations in the amount of soil nitrogen or other nutrient deficiency which has exercised a selective action on plant survival farther within the gypsum area. The leaf nitrogen of all species listed in Table 1 is high in proportion to the low nitrogen content of the soil. Other workers have shown, however, that leaf nitrogen does not necessarily decrease in proportion to nitrogen of the substrate. Table 2 illustrates this point (Gregory, 1937).

There is existing evidence that atmospheric ammonia may be adsorbed on soil colloids (Ingham, 1938), but colloidal matter in the gypsum sand is essentially non-existent. Calcium sulphate itself has an ammonia-fixing capacity, however, apparently preventing the loss of volatile ammonium carbonate by the formation of non-volatile ammonium sulphate (Bear & Workman, 1919). Since plants absorb ammonium salts most readily at an alkaline reaction, the *p*H of the gypsum substrate, 7·5, would favor ammonia absorption. Though the gypsum does not retain well such nitrogen as is produced, plants frequently grow in other soils which are highly inefficient in retaining this element (Shields, 1953). While nitrogen as nitrates may amount to as much as 300 p.p.m. of moist agricultural soil (Nicol, 1934), many such soils, once crops start to develop, are practically devoid of nitrate because of its rapid uptake though the amounts being liberated by soil organisms may be ample for good yields

(Bray, 1945). In nutrient solutions used for experimental work not concerned specifically with nitrogen deficiencies, a total concentration of nitrogen of around 55 p.p.m. is sometimes maintained in the form of nitrates, ammonia or the two combined (Sideris, Kraus & Young, 1937; Hobbs, 1944). Peach trees make satisfactory growth only when the nitrogen content of the nutrient solution is maintained at 60 or more p.p.m. (Cullinan & Batjer, 1943). Leaves of sweet potato plants receiving 10 p.p.m. nitrogen, however, are light green, but few are shed (Leonard, Anderson & Gieger, 1948). In rooted, leafy citrus cuttings transferred to culture solution and given potassium nitrate to supply nitrate in quantities of 1, 2, 3, 5, 10 and 15 p.p.m., growth increases with each successive increment of nitrogen (Haas, 1937).

Precipitation contributes in some degree to the nitrogen supply of all soils. Rain and snow have been found to bring down annually from four (Russell & Richards, 1919) to more than twelve pounds (Wilson, 1921) of nitrogen per acre.

Summary

Total and amino nitrogen determinations were made by micro-Kjeldahl analysis on mature leaves of forty and on the leafless stems of two plant species growing in a drifting gypsum substrate having a concentration of total nitrite and nitrate of from 0·5 to 5 p.p.m. Two series of observations relate leaf nitrogen concentration to leaf structure rather than to the amount of soil nitrogen at the collection site. (1) The total nitrogen content for the dicotyledon leaves, expressed as a percentage of dry weight, ranges from 1·80 in the xeromorphic type leaf of *Lycium berlandieri* var. *parviflorum* to 5·77 in the mesomorphic leaf of *Helianthus annuus* while total nitrogen of the monocotyledons varies from 1·00 in the leafless stem of *Juncus mexicanus* to 1·75 in leaves of *Sporobolus airoides*. While the exact position of an individual species may vary in the list of leaves arranged in order of diminishing nitrogen content at different times of the growing season, both total and amino leaf nitrogen are consistently highest in thin, predominantly mesomorphic leaves of herbaceous species; second highest in the succulent leaf type thickened by a dorsiventral development of water storage tissue and sometimes also by multiple palisade layers; third, in mesomorphic leaves of woody species; fourth, in xeromorphic dicotyledon leaves greatly reduced in size, small celled, and often densely covered with trichomes; and lowest in monocotyledon leaves dominated by conspicuous mechanical tissue. Leaf nitrogen is more or less proportional to the relative leaf volume occupied by living protoplasts. Total nitrogen is in most cases somewhat higher in May than in August, the increase in cell walls and other inert materials apparently diluting nitrogenous constituents as the leaf grows older. Amino and total nitrogen are essentially the same in monocotyledon and other leaves low in nitrogen, but the gap between the two increases with total nitrogen to 1·78 per cent in leaves of *Helianthus annuus*. (2) Variations in leaf nitrogen bear no apparent relation to the concentration of nitrite and nitrate in the gypsum substrate within the range observed. Certain of the leaves highest in nitrogen were collected from parts of the gypsum deposit having a concentration of nitrite and nitrate of only 1 p.p.m. Total leaf nitrogen of *Cucurbita foetidissima*, *Rhus trilobata* and *Atriplex canescens* grown on a range soil having a concentration of inorganic nitrogen of 15 p.p.m. is not significantly greater than total nitrogen of comparable leaves from the same species grown in gypsum sand.

Many sand samples from the interdunal depressions and occasionally those from the dunes are capable of nitrification in ammonium sulphate solution over a period of three months, indicating the presence of a nitrifying microflora. The soluble nitrogen compounds released in the gypsum substrate are spotty in distribution, however, and are apparently utilized, leached or lost by volatilization as rapidly as produced. This assumption would account for the apparently prohibitive deficit of soil nitrogen in an area which supports plants having a moderate leaf nitrogen content.

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MICROSTRUCTURE OF WOOD OF *PODOCARPUS*[1]

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Introduction

A study of microstructural features of the wood of seventy-four of the approximately one hundred and six species of *Podocarpus* was made in an attempt to discover if wood microstructure agrees with the newer taxonomic treatment of the genus. Buchholz (1948) and Buchholz & Gray (1948a) proposed the abolishment of sub-genera listed by Pilger (1926) and the establishment of eight sections for the genus. The treatment is based chiefly upon leaf anatomy. Later detailed papers dealing with the various sections and sub-sections have since appeared (Buchholz & Gray, 1948b, 1948c; Gray & Buchholz, 1948d, 1951a, 1951b), and Gray has been able to continue extending detailed descriptions of species and varieties (1953a, 1953b). Advantage of the newer treatment is apparent when it is realized that leaf anatomy alone delimits all eight sections. Also, from careful studies of geographic distributions of the various sections, together with many other features, the authors proposed possible closer relationships of certain of the sections. In general, the wood analyses made in this study support the newer classification. Certain similarities in wood structure of species of presumably more closely related sections are also disclosed.

Earlier reports on some of the microstructural features of the wood of podocarps have been made with particular reference to those of long established commercial use (Dadswell & Eckersley, 1935; Greguss, 1951; Howard, 1948; Mello, 1950; Moll & Janssonius, 1906; Phillips, 1941; Record, 1949). Phillips, in 1941, reported on eleven species. Greguss, in 1951 reported from Hungary on twenty-three species, including *Prumnopitys elegans* Phillips, now recognized as *Podocarpus andinus* Poeppig ex Endlicher, the plum-fruited yew of Chile, and also another species he calls *P. lycopodioides* of unknown status. No report other than a preliminary one by the writer (Kaeiser, 1950) is known to consider the woods of species from the standpoint of their various sections.

Materials and Methods

The nucleus of the wood collection was that given to the writer by Buchholz from his New Caledonian collections. These have been subsequently added to from many different sources. Acknowledgement for mature wood and twig specimens should be made particularly to the following individuals and institutions: I. W. Bailey, H. E. Dadswell, C. H. Doherty, F. H. Ferreira, Netta E. Gray, L. H. Holdridge, B. Francis Kukachka, H. R. Orman, O. A. Oaks, E. W. J. Phillips, Florencio Tamesis; Chicago Natural History Museum; Conservator of Forests, British Honduras; The Department of Forestry at Quito, Equador; Imperial Forestry Institute, University of Oxford; Ministries of Agriculture of Brazil and of Cuba; New York Botanical Garden; Smithsonian Institution; University of Illinois; and Yale University School of Forestry.

One hundred eighty-six wood specimens were examined in precise transverse, tangential and radial sections. These were sections cut on a sliding microtome approximately twenty microns

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in thickness, stained with safranin and fast green and mounted in canada balsam in the usual manner. Representatives from each of the eight sections have been examined, including the one species of Section Microcarpus (*P. ustus* Brongn. & Gris), and the one species of Section Sundacarpus (*P. amarus* Bl.). Also, species from both sub-sections A and B of Section Stachycarpus have been examined, as well as species belonging to five of six designated sub-sections of Section Eupodocarpus. The exception here is *P. rostratus* Laurent of sub-section E. This species comes from Madagascar and may now be extinct (Gray, 1953b).

A number of the more newly described species have no authentic mature wood specimens for study at present. This is particularly true of a number of Central and South American species. In all cases herbarium twig specimens were sought in order to aid in the wood analyses. It is necessary to point out that some differences in microstructural features may be due to differences in the age of the wood specimens. This has been taken into consideration in making comparisons. For example, the number and arrangement of cross-field pits within each field have been found to differ frequently between young and mature wood. Only mature woods were considered in reporting these features.

Because of the uncertainty and confusion in the names of a large number of specimens that Buchholz and Gray had to examine in their taxonomic treatment, only species approved by them are here recognized. *P. Henkelii*, *P. latifolius* and *P. usambarensis* are three examples of species often confused with other species. Any wood specimens of uncertain origin were eliminated from the present study.

The New Caledonian wood specimens provided by Buchholz include *P. ustus*, the one species of Section Microcarpus, and whose mature wood is still to be described (the writer's specimen is from an herbarium specimen ex Mus. Paris); and four new species and one variety named by him, all hitherto undescribed in their wood anatomy: *P. Comptonii*, *P. distichus*, *P. distichus* var. *maialis*, *P. palustris*, and *P. sylvestris*. Twig specimens of nine new South American species (not including new varieties) described by Buchholz and Gray have also been examined. These include *P. Cardenasii*, *P. magnifolius*, *P. pendulifolius*, *P. Pittieri*, *P. Reichei*, *P. Rusbyi*, *P. Standleyi*, *P. Steyermarkii* and *P. tepuiensis*. Authentic mature wood samples are yet to be collected for these, as well as for *P. decipiens* N. Gray.

Keysort punch cards of the writer's own design have been used for all specimens, after entry in a journal book, and these have been of much assistance in assorting data. Samples of mature wood and, in many cases, twig specimens as well of forty-four different species have been examined. Twig specimens only of an additional thirty species have also been examined. All of the species are listed below in their respective Sections:

Section 1 — Dacrycarpus Endl.

P. dacrydioides A. Rich.	M
P. imbricatus Bl.	M
P. vieillardii Parl.	M

Section 2 — Microcarpus Pilg.

P. ustus Brongn. & Gris	Y

Section 3 — Nageia Endl.

P. blumei Endl.	M
P. nagi (Thunb.) Pilg.	Y
P. motleyi (Parl.) Dummer	M
P. wallichianus C. Presl.	M

Section 4 — Afrocarpus Buchh. & Gray

P. falcatus (Thunb.) R. Br.	M
P. gracilior Pilg.	M
P. usambarensis Pilg.	M

Section 5 — Polypodiopsis Bertrand

P. comptonii Buchh.	M
P. minor Parl.	M
P. rospigliosii Pilg.	Y
P. palustris Buchh.	M
P. vitiensis Seem.	M

Section 6 —Sundacarpus Buchh. & Gray

P. amarus Bl.	M

Section 7 — Stachycarpus Endl., in part

Sub-section A — Euprumnopitys Buchh. & Gray

P. andinus Poepp.	M
P. ferrugineus Don	M
P. harmisianus Pilg.	Y
P. montanus (Willd.) Lodd.	M
P. montanus var. *densifolius* (Kunth.) Buchh. & Gray	Y
P. montanus var. *meridensis* Buchh. & Gray	Y
P. standleyi Buchh. & Gray	Y
P. spicatus R. Br.	M
P. utilior Pilg.	Y

Sub-section B—Idioblastus Buchh. & Gray

P. distichus Buchh.	M
P. distichus var. *maialis* Buchh.	M
P. ferruginoides Compt.	M

Section 8 — Eupodocarpus Endl. M

Sub-section A

P. elongatus (Ait.) L'Herit.	M
P. henkelii Stapf.	M
P. latifolius (Thunb.) R. Br.	M
P. milanjianus Rendle	M

Sub-section B

P. elatus R. Br.	M
P. macrophyllus D. Don	M
P. macrophyllus var. *Maki* Sieb.	M
P. neriifolius D. Don	M
P. neriifolius var. *Degeneri* Gray	Y
P. novae-caledoniae Vieill.	Y
P. philippinensis Foxw.	M
P. polystachus R. Br.	M
P. rumphii Bl.	M
P. sylvestris Buchh.	M

Sub-section C

P. angustifolius Grise	M
P. aristulatus Parl.	Y
P. buchii Urban	Y
P. buchii var. *latifolius* Florin	Y
P. cardenasii Buchh. & Gray	Y
P. coriaceus L. C. Rich.	M
P. ekmanii Urban	Y
P. glomeratus D. Don	M
P. guatemalensis Standl.	M
P. guatemalensis var. *Allenii* (Standl.) Buchh. & Gray	Y
P. lambertii Klotzsch	M
P. lambertii var. *transiens* Pilg.	Y
P. leoni Carabia	Y
P. magnifolius Buchh. & Gray	Y
P. matudai Lundell	Y
P. matudai var. *macrocarpus* Buchh. & Gray	Y
P. oleifolius D. Don	M
P. oleifolius var. *costaricensis* Buchh. & Gray	Y
P. oleifolius var. *macrostachyus* (Parl.) Buchh. & Gray	M
P. oleifolius var. *trujillensis* Buchh. & Gray	Y
P. parlatorei Pilg.	Y
P. pendulifolius Buchh. & Gray	Y
P. pittieri Buchh. & Gray	Y
P. purdieanus Hook.	Y
P. reichei Buchh. & Gray	Y
P. roraimae Pilg.	Y
P. rusbyi Buchh. & Gray	Y
P. salignus D. Don	M
P. sellowii var. *angustifolius* Pilg.	Y
P. sprucei Parl.	Y
P. steyermarkii Buchh. & Gray	Y
P. tepuiensis Buchh. & Gray	Y
P. urbanii Pilg.	Y

Sub-section D

P. acutifolius Kirk	Y
P. alpinus R. Br.	M
P. gnidioides var. *caespitosus* Carr.	Y
P. hallii Kirk	M
P. nivalis Hook.	Y
P. nubigenus Lindley	M
P. totara D. Don	M

Sub-section E (no specimen)

Sub-section F

P. decipiens N. Gray	Y
P. longifoliolatus Pilg.	M
P. salomoniensis Wasscher	M

M=mature wood; Y=young wood only.

The approximate numbers of species described and recognized as valid are listed below by sections, together with total numbers of species represented by wood specimens assembled by the writer;

Section	Approx. No. of species described	Species represented by mature wood	Species represented by twig wood only
Dacrycarpus	10	3	0
Microcarpus	1	0	1
Nageia	7	3	1
Afrocarpus	6	3	0
Polypodiopsis	5	4	1
Sundacarpus	1	1	0
Stachycarpus			
Sub-section A	7	4	3
Sub-section B	3	2	0
Eupodocarpus			
Sub-section A	6	4	0
Sub-section B	21+	7	1
Sub-section C	27	7	19
Sub-section D	7	4	3
Sub-section E	1 (May be extinct)	0	0
Sub-section F	4+	2	1
Total	106+	44	30

Observations

Table 1 gives the microstructural features of the woods according to sections. Presence or absence of xylem parenchyma is one of the striking differences noted. Xylem parenchyma is either absent (or very sparse), or relatively abundant for gymnosperms within each section, with the exception of *P. andinus* of Stachycarpus Sub-section A, which, unlike other associated species of this group, has abundant xylem parenchyma present. Xylem parenchyma is observable in very early wood, and, in specimens where found to be present, also persists in relatively the same amounts in mature wood.

Relative ray heights (tangential aspect) are given. Parentheses around the "×" are used if but a part of the group shows the type indicated being present.

Horizontal wall thickness refers to the relative thickness of the walls of ray parenchyma with respect to contiguous tracheid walls as observed in radial sections of early (spring) wood. Relatively few species show this characteristic, and these are confined to Section Eupodocarpus (parts of Sub-sections B and C and all species of Sub-section D examined).

The term "pits" of ray cells refers to primary pit fields in the horizontal walls of ray parenchyma cells, and are listed as present in Section Eupodocarpus, with part of Sub-sections A, B and C, and all species of Sub-section D having them.

Indentures in the end walls of ray cells are, likewise, confined to Section Eupodocarpus (parts of Sub-sections A, B and C, and all species of Sub-section D examined).

Averages of height of ray cells are based on averages of measurements in tangential view of ten random counts for each species.

The report on cross-field pits is based upon studies of cross-fields of early (spring) wood in all cases. Again parentheses are used if the characteristic is not present for all species. When frequently empty cross-fields (no pits) were encountered, as in Sections Nageia, Afrocarpus and portions of Eupodocarpus, they were recorded. When more than one cross-field pit was frequently present per field, this condition was also indicated. Only one cross-field pit per field was consistent for Sections Sundacarpus, Stachycarpus and Eupodocarpus. Relatively larger sized cross-field pits (one-half height of field to filling field) were noted in Sections Microcarpus, Nageia, Sundacarpus and part of Sub-section A of Section Stachycarpus. Cross-field pit apertures refer to ranges in length of pits based upon ten random counts in early wood for each specimen.

TABLE 1—MICROSTRUCTURAL FEATURES OF WOOD OF THE EIGHT SECTIONS AND SUB-SECTIONS OF *PODOCARPUS*

Sections	Xylem parenchyma		Rays					Cross-field pits								Tracheids		
	Present	Absent	Low Medium High	Horiz. walls Rela. thick	"Pits"	Indentures	Ave. ray cell ht. (microns)	Empty fields	Large pits	Taxodioid	Cupressoid	Piceoid	Frequently 2 or more per field	Half ht. of field to filling field	Pit aperture L. (microns)	Frequent tangential pitting	Frequent radial pit twinning	Radial pits crowded (touching)
1. Dacrycarpus		×	×××				18			×	×	×	1 or 2		6-9		×	×
2. Microcarpus		×	×				24		×	×			×	×	9-15			×
3. Nageia	×		×				21	×	×	×	×		1 or 2	×	9-15		×	
4. Afrocarpus	×		×××				21	×		×	×		1 or 2		6-9	×	×	×
5. Polypodiopsis	×		×				21			×	×		×		9-15	×	×	(×)
6. Sundacarpus		×	××				24		×	×				×	9-18		×	
7. Stachycarpus																		
Sub-sec. A		×	×××				21		(×)	×	×			(×)	6-18			
Sub-sec. B		×	×				21			×	×				6-12			
8. Eupodocarpus																		
Sub-sec. A	×		×××		(×)	(×)	21	(×)		×	×				6-15	×	×	×
Sub-sec. B	×		××(×)	(×)	(×)	(×)	21	×		×	×	×			9-12	×	×	×
Sub-sec. C	×		××	(×)	(×)	(×)	21			×	×				9-12	×	×	×
Sub-sec. D	×		××(×)	×	×	×	21	×		×	×	×			6-12	×	×	×
Sub-sec. E																		
Sub-sec. F	×		××				24	(×)		×	×	×			6-12	×		×

Tracheid characteristics reported include relative frequency of tangential pitting, as observed in Sections Afrocarpus, Polypodiopsis and Eupodocarpus; relatively frequent twinning of bordered pits in radial walls (Sections Dacrycarpus, Nageia, Afrocarpus, Polypodiopsis, Sundacarpus and Sub-sections A, B, C, and D of Section Eupodocarpus); and

The key given below shows how, by using four microstructural characteristics of the wood, most the the Sections may be delimited. All of these characteristics persist in both young and mature wood wherever examined, although the key is based entirely upon mature wood specimens, except for *P. ustus*, the one species of Section Microcarpus.

1. Xylem Parenchyma Absent or Sparse	
A. Large Cross-field Pits	
1) Radial pits on tracheids "crowded"	Microcarpus (*P. ustus*)
2) Radial pits on tracheids not "crowded"	
a) Large, and taxodioid cross-field pits only	Sundacarpus (*P. amarus*)
b) Large, taxodioid and cupressoid cross-field pits	Stachycarpus Sub-sec. A, in part including: (*P. spicatus, P. Harmsianus, P. utilior*)
B. No Large Cross-field Pits	
1) Radial pits on tracheids "crowded"	Dacrycarpus
2) Radial pits on tracheids not "crowded"	Stachycarpus Sub-sec. A, in part; Sub-sec. B
2. Xylem Parenchyma Abundant for Conifers	
A. Large Cross-field Pits	Nageia
B. No Large Cross-field Pits	
1) Rays low, medium, and high	Afrocarpus; Eupodocarpus; Sub-sec. A
2) Rays low and medium	Eupodocarpus, Sub-sec. B, C, D, F
3) Rays low only	*P. andinus* of Stachycarpus, Sub-sec. A; Polypodiopsis

the comparative crowding of radial bordered pits (a condition present in Sections Dacrycarpus, Microcarpus, Afrocarpus, a portion of Section Polypodiopsis, and all species examined of Section Eupodocarpus).

Table 2 gives a comparison of leaf structure in the eight Sections with some selected characteristics of their woods. It should be noted that the wood samples analyzed are random samples and may not be representative of the extremes of variation existing in the various groups. The need for more extensive collections to include many samples from known regions in different parts of the plants and from specimens growing under different environmental conditions is apparent. On the basis of the limited type of wood samples that were available, however, the outstanding microstructural features of the Sections appear to be those listed in the table.

Discussion

Sections Dacrycarpus and Microcarpus are considered probably the most primitive (Buchholz & Gray, 1948a) with their scarcely developed foliage (Table 2). Xylem parenchyma is absent in members of both of these sections. The next three sections, Nageia, Afrocarpus, and Polypodiopsis, have amphistomatic leaves (stomata on both upper and lower surfaces of leaves). Members of all three sections have xylem parenchyma. Afrocarpus and Polypodiopsis are considered closely related, having a common precursor, with Polypodiopsis being the older of the two sections. It will be noted from the tables that in both of these sections the large type of cross-field pits are absent, although present in Nageia. Nageia wood is similar to the one species of Microcarpus (*P. ustus*) in this respect.

TABLE 2—COMPARISON OF LEAF STRUCTURE WITH SOME MICROSTRUCTURAL FEATURES OF WOOD IN THE EIGHT SECTIONS OF *PODOCARPUS*

Section	Leaf structure			Microstructural features of wood										
				Parenchyma		Cross-field pits				Tracheids		Rays		
	Scarcely developed leaves	Leaves amphistomatic	Leaves hypostomatic	Xylem parenchyma present	Xylem parenchyma absent or sparse	Large pits	Taxodioid	Cupressoid	Piceoid	Frequent tangential pitting	Radial pits crowded (touching)	Low	Medium	High
1. Dacrycarpus	×				×		×	×	×		×	×	×	×
2. Microcarpus	×				×	×	×				×	×		
3. Nageia		×		×		×	×	×				×		
4. Afrocarpus		×		×			×	×		×	×	×	×	×
5. Polypodiopsis		×		×			×	×		×	(×)	×		
6. Sundacarpus			×		×	×	×					×	×	
7. Stachycarpus														
Sub-sec. A			×		×	(×)	×	×				×	×	×
Sub-sec. B		×			×		×	×				×		
8. Eupodocarpus														
Sub-sec. A			×	×			×	×		×	×	×	×	×
Sub-sec. B			×	×			×	×	×	×	×	×	×	(×)
Sub-sec. C			×	×			×	×		×	×	×	×	
Sub-sec. D			×	×			×	×	×	×	×	×	×	(×)
Sub-sec. E			×											
Sub-sec. F			×	×			×	×	×	×	×	×	×	

Section Afrocarpus and Sub-section A of Eupodocarpus, which are listed together in the key, are the two groups found in Africa. Analyses of the microstructural features of the woods of the two groups did not disclose any consistent differences. All specimens of *P. falcatus*, *P. gracilior* and *P. usambarensis* of Section Afrocarpus showed typically both cupressoid and taxodioid cross-field pits in early wood. In Sub-section A of Section Eupodocarpus *P. elongatus* specimens of wood examined disclosed mostly cupressoid cross-field pits, as did *P. Henkelii*, *P. milanjianus* and *P. latifolius*. However, taxodioid pits are present in all four species so that this characteristic is considered too uncertain to be of diagnostic value.

Sub-section B of Stachycarpus is also amphistomatic and is regarded as a possible point of origin for the entire section in the south Pacific land areas. Members of this group, however, lack xylem parenchyma.

Sub-section A of Stachycarpus, Sections Sundacarpus and Eupodocarpus are all hypostomatic (stomata confined to the lower epidermis). Sub-section A of Stachycarpus also lacks abundant xylem parenchyma (except *P. andinus*), as does Sundacarpus. Members of Eupodocarpus, however, have these elements.

Stachycarpus may have been derived from Sundacarpus. Both sections lack xylem parenchyma (except *P. andinus*), and they also lack frequent tangential pitting and crowding of radial pits on tracheids.

Eupodocarpus is regarded as the most recent in origin and contains members with foliage most similar to Sundacarpus. It differs from Sundacarpus, however, in having xylem parenchyma, in lacking large cross-field pits, and in possessing frequent tangential pitting and frequently crowded radial pits on tracheids. If Eupodocarpus was derived from any or all of the sections possessing xylem parenchyma (Nageia, Afrocarpus, Polypodiopsis), Sundacarpus and Stachycarpus must have arisen at the same time and from the same source, probably a type of Polypodiopsis.

The xylem parenchyma of *P. andinus* of Stachycarpus, Sub-section A, may indicate that this entire section is one in which the feature was subsequently lost or was a variable character of its precursor. Of the three species in this sub-section having large cross-field pits, *P. Harmsianus* and *P. utilior* are most closely related; *P. spicatus*, on the basis of leaf structure, would appear to be no more closely related to them, however, than to *P. ferrugineus* which lacks large cross-field pits in its wood. Possession of large cross-field pits in these members, however, may link this group more closely with Sundacarpus from which it is possibly derived.

Summary

1. Microstructural features of the woods of seventy-four species of *Podocarpus* are presented according to Sections and Sub-sections.

2. Significant microstructural features which separate most of the Sections are limited to: (a) presence or absence of xylem parenchyma; (b) types of cross-field pits; (c) crowding or non-crowding of radial pits of tracheids; and (d) relative heights of rays.

3. *P. andinus* of Section Stachycarpus is an unusual species in that, unlike associated species of the Section, it has relatively abundant xylem parenchyma.

4. In the key presented, Sections Afrocarpus and Sub-section A of Section Eupodocarpus are not delimited.

5. Wood characteristics are offered as a possible basis for showing relationships of certain of the sections.

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DEVELOPMENTAL ANATOMY OF CHICORY — THE ROOT[1]

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Chicory (*Cichorium intybus* L.) is a plant of some economic importance because of its use in the beverage industry. A study of its developmental anatomy was, therefore, thought desirable. Tenopyr (1918) made a study of cell shape in the leaves. Lee (1914) mentioned the cotyledonary bundles. Stout and Boas (1921) made a statistical study of the number of flowers per head. Grier (1919) noted variation in the flowers. Makowetsky (1929) studied the chromosomes and Krüger (1898) reported on the stem structure. Only passing reference is made to chicory in other reports. It is planned to make a co-ordinated study of all parts of the plant, beginning with the root.

Materials and Methods

A strain of chicory labeled Accession 6, Number 15, was obtained from Mr. Harold

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Kohls of the Farm Crops Department and used throughout the study. Fruits were planted in soil in clay saucers. The seeds germinated readily and the growth of the seedlings was rapid; plants started on March 9th, for example, were transplanted to the field on May 27th and flowered on September 27th, after having been brought back into the greenhouse.

Seedling roots were killed and fixed in Craf 2. Tertiary butyl alcohol was used as a dehydrating agent for the material before imbedding in paraffin. Conant's quadruple stain schedule was used for most of the slides. Photographs were made either with a Leitz Makam or with a Kodak 35 mm. adapter. Some of the photographs were taken by Mr. Philip Coleman.

FIGS. 1-6 — Seedling development. Fig. 1. Seven days. × ⅓. Fig. 2. Fourteen days. × ⅓. Fig. 3. Twenty-two days. Note first true leaf. × ⅓. Fig. 4. Thirty-four days. Note second true leaf. × ⅓. Fig. 5. Forty-six days. Note three true leaves. × ⅓. Fig. 6. Seventy-four days. Note five true leaves. × ⅛.

Observations

SEEDLING DEVELOPMENT — The fruit of chicory is an achene. Under suitable conditions for germination the primary root breaks through the pericarp wall and grows downward. The hypocotyl, that portion of the plant between the cotyledons and the root and a large part of the "root" of commerce, elongates rapidly, carrying the stem tip, cotyledons and the split pericarp upward. The hypocotyl exceeds the primary root in length until the early "rosette" stage of the leaves. The first true leaves are entire; the later formed ones are lobed. The stem grows slowly. One of the results of this habit, as indicated above, is that the first true leaves are arranged closely together forming a rosette. Figs. 1-6 illustrate the general development of the seedling. The hypocotyl and root are perennial while the stalk, bearing some of the leaves and the inflorescences, is annual. The root is usually spindle-shaped (Fig. 7) and can become quite large.

ANATOMY OF THE COTYLEDONS — The lower part of the hypocotyl is root-like in that it has radially arranged vascular tissues. The upper part is stem-like with collateral vascular tissues. The transition region is in the upper part of the hypocotyl.

In the upper hypocotyledonary part of the embryo, there is a pith surrounded by a ring of procambial tissue. Proceeding apically, one notes that this ring consists of six distinct procambial strands. Those two at either end, lying in the mid-line of the cotyledons, become united further up to form the main bundles of the cotyledons. Because of their origin, they are known as "double" bundles. A cross-section at this point reveals two "double" bundles and two "single" bundles. At the very base of the cotyledons, each of the "single" bundles has been replaced by two strands, one of which "enters" each cotyledon. Thus each cotyledon has three bundles, a larger central one and two smaller, lateral ones. The connection of the "double" bundles with the vascular part of the hypocotyl is shown in Fig. 8. The vessel elements of the bundles have

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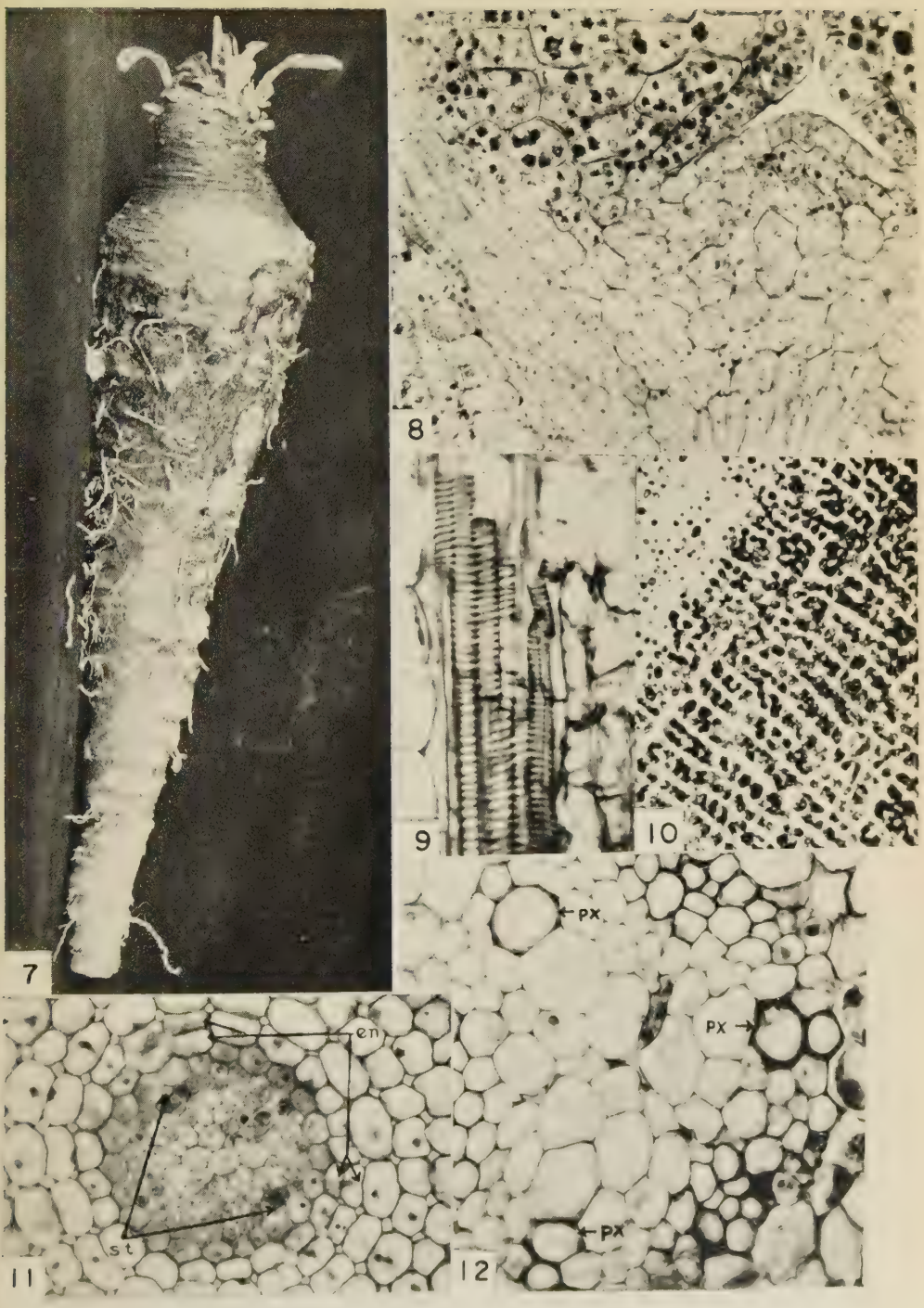
FIGS. 7-12 — Fig. 7. Mature root. × ¼. Fig. 8. Procambial strand into cotyledon. Note one-layered tunica and the underlying corpus. × 290. Fig. 9. Detail of vessels in the cotyledon. × 267. Fig. 10. Inulin in the cotyledon cells. × 290. Fig. 11. First phloem elements *s.t.* Note partially biseriate endodermis *en.* × 267. Fig. 12. Triarch root with three primary xylem groups *px.* × 290.



Figs. 7-12.

spiral and annular thickenings (Fig. 9). The epidermis of the cotyledons has stomates; a one or two-layered palisade is evident; and most of the cotyledon cells contain reserve food in the form of inulin. This has been coagulated by the chemicals in the paraffin schedule and is seen in Fig. 10. Inulin crystals can be seen in the cells of alcohol-preserved roots by treatment with thymol and sulphuric acid.

Primary Tissues of the Root — A root cap is present, even in the embryo. It originates from the meristematic protoderm.

The epidermis, likewise differentiated from the protoderm, is uniseriate and without a cuticle. It sloughs off after secondary growth is established.

The cortex is commonly seven cells deep except opposite the primary phloem. At these two places the cortex is eight cells deep because the endodermis is biseriate there (Fig. 11). This condition of the endoderms may be noted prior to the differentiation of the primary phloem elements. The innermost cortex has many intercellular spaces but the outer layers are compact. Like the cells of the cotyledons, the cortical cells are conspicuous for their high inulin content. Most of the cortical cells lie in radial sheets. Since the peripheral layer frequently has cells half the size of the cells further toward the center, it can be said that some of the cortical cells are capable of anticlinal divisions. The result of this activity could be to increase the diameter of the root while it is still in the primary condition.

The promeristem at the root tip is composed of tightly-packed cells and a stele is not distinguishable. The first and most peripheral layer of the stele to be noted, in serial sections, is the uniseriate pericycle. Two derivatives of this layer are lateral root primordia and phellogen, both to be mentioned later.

The primary phloem consists of sieve tubes, companion cells, other parenchyma cells and an occasional laticifer. It is convenient to designate the first phloem as protophloem and that formed later as metaphloem although it is difficult to delimit them visually with any great degree of exactitude. The protophloem is the first vascular tissue to differentiate. This is evidenced by the appearance of two cells which are thin-walled and angular, in cross-section, one on each side of the stele and up against the pericycle (Fig. 11). These cells appear to be sieve tubes. Usually one or two nearby cells differentiate on each side into sieve tubes to complete the protophloem as seen in cross-section. No companion cells are found. The metaphloem is composed of sieve tubes and parenchyma cells, including companion cells. The total amount of metaphloem is larger than that of the protophloem.

The usually diarch primary xylem lignifies after the primary phloem has differentiated. Occasionally a triarch root is encountered (Fig. 12). In such cases, a biseriate endodermis overlies each phloem group. In diarch roots, the exarch xylem lignifies centripetally and completion of this process results in a protostele. In the material studied, one protoxylem point was capped by a single tier of xylem cells and the other point by two tiers. The metaxylem cells are larger in diameter and lignify later than the protoxylem cells. The wall thickenings of the primary xylem are annular, helical and scalariform. The total amount of primary tissues is small and there is probably some correlation between this fact and their short span of usefulness.

Lateral roots form endogenously from dividing cells of the pericycle at the protoxylem points (Fig. 13). Potentialities evidently exist in the cells of the primordium for the formation of a root

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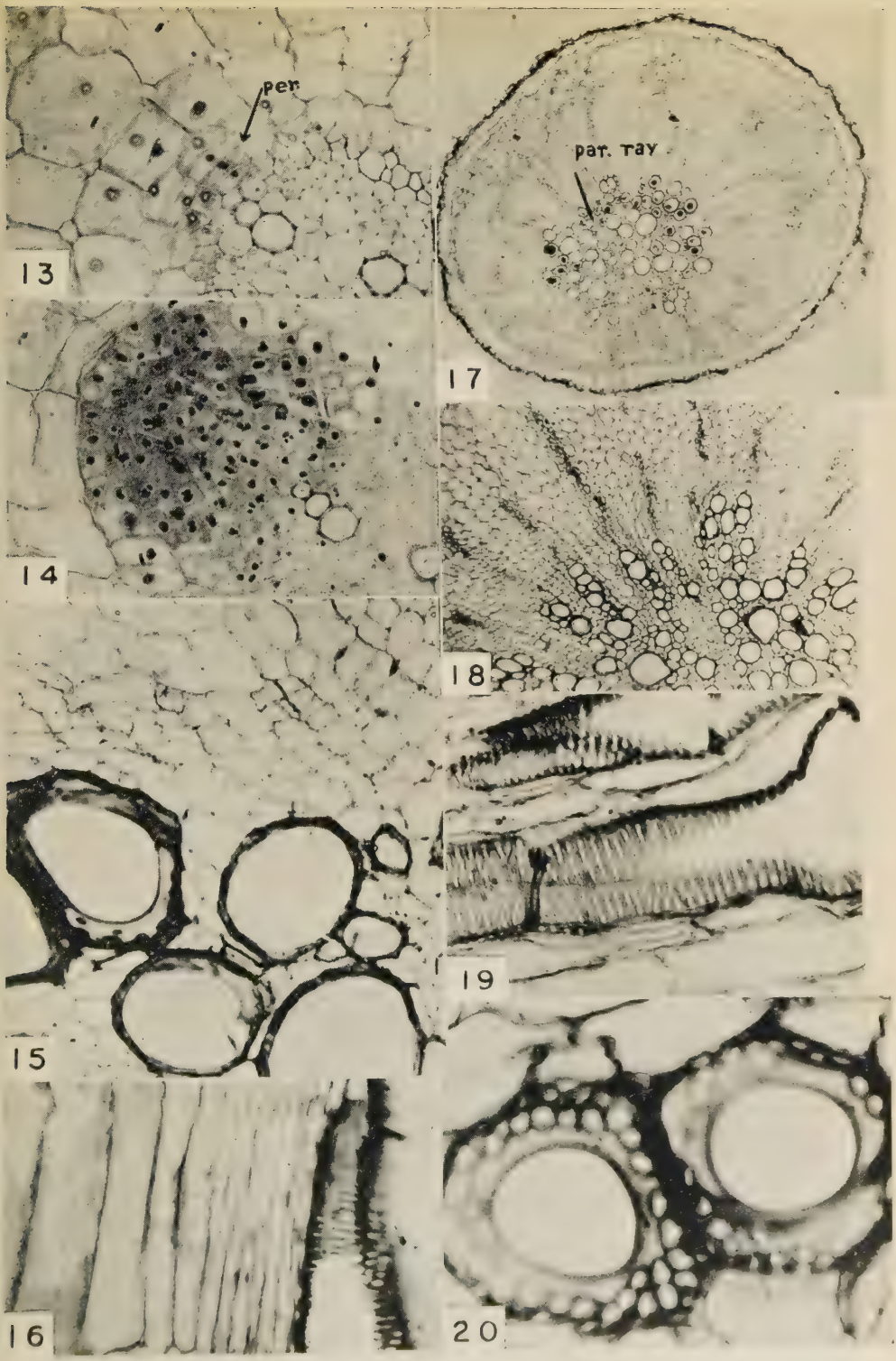
Figs. 13-20 — Fig. 13. Initiation of lateral root primordium by divisions in cells of the pericycle *per.* × 560. Fig. 14. Lateral root primordium formed opposite protoxylem pole. × 560. Fig. 15. Cambium and secondary tissues. × 290. Fig. 16. Fusiform initials in cambium. × 272. Fig. 17. Cross-section mature root. Note periderm and ray of parenchyma cells in xylem *par. ray.* × 16. Fig. 18. Mature root detail with spoked arrangement of vascular elements in phloem. × 272. Fig. 19. Type of pitting common in vessel segments of secondary xylem. × 290. Fig. 20. Pitted rims in end walls of secondary xylem vessels. × 720.



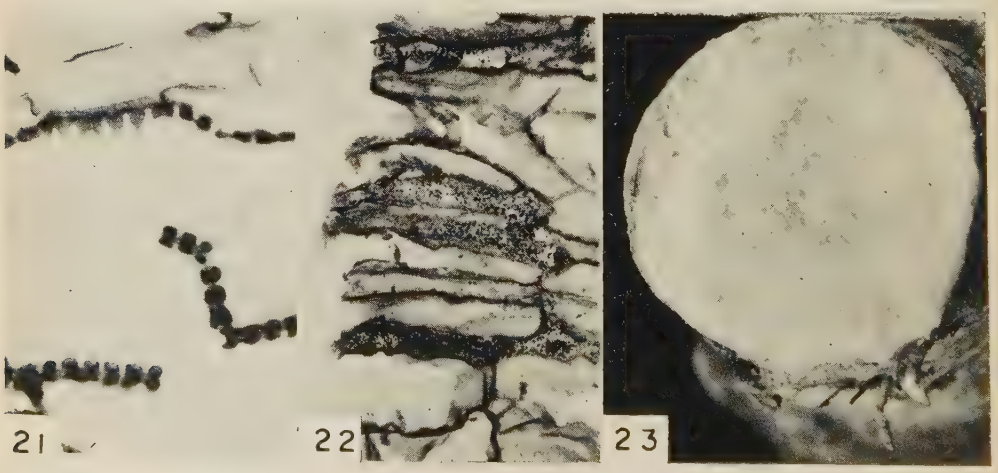
Figs. 13-20

cap, epidermis, apical meristem, cortex and stele. As the primordium grows, the cortical cells of the primary root are displaced (Fig. 14). The endodermis surrounds the primordium when young, appears on the sides later but cannot be followed as a unit, due to rupturing, when the primordium is near the peripheral edge of the cortex. Two rows of lateral roots are matured.

Secondary Tissues — These assume great significance since the " root " of commerce is practically all secondary in nature. The procambium in the stele, between the radially-arranged primary vascular tissues, becomes active and forms a cambial zone which, in turn, originates the secondary tissues. The actual, activated cells are not at first clearly distinguishable but they soon form a definite tier (Figs. 15, 16). A continuous band later results due to activity of the pericycle. The cambium formed from the pericyle produces mostly ray cells and hence little vascular tissue develops over the protoxylem poles. As a result, a rather persistent ray of parenchyma can be noted running through the secondary xylem from each protoxylem pole (Fig. 17). It was observed that when the secondary tissues became evident, the cells of the cortex and the epidermis lose their turgidity and collapse. Later these two tissues slough off.

The secondary xylem, consisting of parenchyma cells and vessel segments, forms on the inner face of the vascular cambium. At first the two kinds of cells are interspersed but later the parenchyma is laid down in rays (compare Figs. 17, 18). The secondary xylem vessels have a variety of pit types on their lateral walls. They range from oval and opposite to scalariform to scalariform-reticulate. The oval, opposite type is most common (Fig. 19). Fig. 21 is a lateral view of the vessel pits. Pitted rims encircle the simple perforations between two contiguous vessel segments (Fig. 20). It was noted that some of the perforations were of the scalariform type.

The secondary phloem is composed of parenchyma cells (including companion cells), sieve tubes and laticifers; the first elements of this secondary tissue frequently appearing before the metaxylem has lignified. Ray initials produce rather wide bands or rays of parenchyma tissue and fusiform initials form rather narrow tiers of vascular elements and laticifers. As a result, the phloem conducting elements and their associated cells take on a spoked appearance (Figs. 17, 18). Three other important facts about the secon-



Figs. 21-23 — Fig. 21. Side view of bordered pits in secondary xylem vessels. × 170. Fig. 22. Laticifers in phloem. × 170. Fig. 23. Latex exuding from young secondary phloem laticifers. × 1½.

dary phloem are worthy of note. One is that this tissue occupies a greater area of the mature root than does the secondary xylem. Secondly, the older and more peripherally placed phloem becomes quite spongy as compared to the younger phloem. Thirdly, the cells of the vascular areas are smaller in diameter than the parenchyma cells of the phloem rays.

Laticifers were not seen in the embryo but may be present in small numbers in the primary phloem after the seed germinates. They are most numerous, however, in the vascular portion of the youngest secondary phloem tissue. Fig. 23 is a view of the latex as it exuded from the laticifers in the young phloem tissues. When examined in longitudinal section, the laticifers are seen to be of the articulated anastomosing type (Fig. 22). The anastomoses seem to be formed by the dissolution of the walls of two contiguous laticifers. The latex itself is white and viscid and, under the microscope, has inclusion bodies in it of various sizes.

Older roots are protected by a periderm, a few cells thick in cross-section. The periderm is derived from a phellogen of pericyclic origin.

Discussion

In a study of cell shape in chicory leaves, Tenopyr (1918) found that the first true leaves are entire, the later ones lobed, a fact also noted in this study. The three cotyledonary bundles seem to be also characteristic of the Russian dandelion (Artschwager, 1943) and of *Lactuca* (Port, 1937). Lee (1914) mentions that only one bundle connects the cotyledon with the hypocotyl of chicory but the material used in this study has one large and two small bundles. The cotyledon midribs are double bundles here as in *Helianthus* and *Arctium* (Siler, 1931). Siler (1931) found palisade layers and stomates in the cotyledons of *Helianthus* as is the case in chicory. The abundant inulin found in the plant is a characteristic of the compositae (Solereder, 1908) although it is found in other families as well.

The usual diarch condition as found in chicory is common, as for example in tragopogon (Havis, 1935). The partially biseriate endodermis of chicory is also characteristic of *Lactuca sativa* (Port, 1937), *Cynara scolymus* (Phillips, 1937), *Parthenium argentatum* (Artschwager, 1943), *Helianthus annuus* (Siler, 1931), *Calendula officinalis* and *Zinnia elegans* (Chauveaud, 1911), but is not found in the Russian dandelion (Artschwager, 1943). The Russian dandelion and *Parthenium argentatum* agree with chicory in that all have a parenchymatous center to the diarch plate in the hypocotyl. The dandelion, chicory and *Lactuca* are all similar in that the epidermis and the cortex slough off. In *Parthenium*, on the other hand, the cortex is persistent, even in old roots. In the usual root pattern, according to Esau (1953) the primary phloem differentiates prior to the primary xylem. This was found to be the case in chicory. Faust (1917) noted the opposite sequence in *Balzamorrhiza*.

Lateral roots originate from the cells of the pericycle, opposite the protoxylem points, as was also found in the Russian dandelion but in *Daucus*, Esau (1940) finds that they arise on either side of the two primary phloem groups. Hayward (1948) found that *Lactuca sativa* agrees with *Daucus* in this regard.

In respect to the secondary tissues, agreement was noted with the Russian dandelion on the following main points — the small xylem core and the general composition of the xylem (vessels and parenchyma). In both *Daucus* and the Russian dandelion vessels were found in the secondary xylem with scalariform pits on their lateral walls. These are also found in chicory but those with oval, opposite pits predominate. *Lactuca* (Port, 1937) agrees with chicory in possessing prominent parenchyma rays opposite the protoxylem points.

The laticifers in the primary and secondary phloem are characteristic of many of the compositae (Solereder, 1908). Artschwager (1943) found them in the pericycle of the Russian dandelion as well as in the phloem and this is also true of *Lactuca* (Port, 1937). In the Russian dandelion the laticifers decrease in numbers in the secondary phloem peripherally as in chicory.

Summary

The seedling development was followed until the early rosette stage and the relationships of the hypocotyl, transition region and the cotyledons were examined. The developmental anatomy of the root was followed in both the primary and secondary conditions. The epidermis is derived from the protoderm. The outer cortical cells are meristematic and contribute to the diameter of the primary root. Both epidermis and cortex slough off. The endodermis is biseriate over the primary phloem groups. The pericycle gives rise to the periderm, to lateral root primordia and completes the ring of cambium over the protoxylem poles. Primary phloem differentiates before the primary xylem does but is composed of a relatively small number of cells when compared to the large amount of secondary phloem. Primary xylem is usually diarch but triarch xylem has been found. The mature root consists mainly of secondary tissues with the upper portion of the " root " being the hypocotyl. Two types of cambial initials are present: one type forms cells which become parenchyma cells in both xylem and phloem and the other type forms cells which become vascular elements, parenchyma, and laticifers in the phloem and vessel segments and parenchyma in the xylem. A " spoked " condition can be seen in the phloem because of the rather sharp delimitation of the functions of the two types of cambial initials. A rather persistent parenchyma ray is found in the xylem opposite each protoxylem point. The laticifers are located mainly in the young, secondary phloem. They are of the articulated anastomosing type. The periderm forms from a pericyclic phellogen and is comparatively thin.

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THE ORGANIZATION AND INTER-RELATIONSHIPS OF THE CARPOSPOROPHYTES OF LIVING FLORIDEAE

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One of the distinctive features which separate the Florideae so sharply from the algae of other divisions is the existence in the life-history of the phase known as the carposporophyte. This structure develops from the zygote, but, unlike the zygote of those other algae, the female cell of which is fertilized *in situ*, it develops immediately in the Florideae and while still attached to the gametophyte. From the zygote grow filaments, which whatever degree of differentiation they may show, ultimately bear carposporangia. In addition to the initiation of the carposporophyte fertilization *may* also set in motion correlated development of cells of the gametophyte, neighbouring the carposporophyte, the resulting " tissue " having either nutritive or protective functions[2]. In many instances, the spore-producing filaments and this post-fertilization " tissue " of the gametophyte together make a physiological unit and a morphological whole. Thus it is apparent that while in some Florideae post-fertilization structures are relatively simple, in others they show considerable differentiation and elaboration.

The small size of the structures involved has resulted in slow progress being made in the accumulation of information. The reading of the relevant literature shows that there are many important families in the Florideae for which no information regarding the post-fertilization development is available or is too incomplete to be of use. A knowledge of the immediate post-fertilization changes are highly important but are unknown in many genera particularly those of the most complex of the Gigartinales (*sensu* Kylin).

At the same time the great variety of detail in the post-fertilization changes has hindered the interpretation of the information and also attempts to understand the organization and interrelationships of the species investigated. However, the time now seems ripe for such a review to be attempted on the basis of the information available in order to indicate the way along which further work should proceed. As terminology is dependent on interpretation, this is considered where relevant. Finally the implication on questions of classification of the conclusions reached regarding inter-relationships of the types recognized is considered briefly.

Terminology

Before starting on the review of the organization of the carposporophyte it is necessary to state clearly what is meant by the term *carposporophyte*. This term was first used by Janet (1914) and was defined by Church (1919) as " the second parasitic individual or generation " — " prevailingly, though by no means necessarily, diploid in its nuclear organization ". A more recent definition by Smith (1944, p. 18) is that it consists of " the gonimoblast filaments and the carposporangia borne on them, plus the carpogonium or the auxiliary cell producing the gonimoblast filaments ". Another term in general use and apparently considered by some writers as synonymous with carposporophyte is *cystocarp*. In fact, Smith on the same page as carpo-

2. In some instances, special nutritive and protective " tissue " is present before fertilization.

1. Paper read at a meeting of Section K of the British Association for the Advancement of Science at Liverpool, September 4, 1953.

sporophyte defines this structure as " the fruit resulting from the fertilization in Rhodophyta " — " in Florideae it consists of carpospores, gonimoblast filaments and the cell from which they grow ". The term *cystocarp* was used first by Agardh (1844) in place of the earlier term capsule and that investigator probably had algae such as *Polysiphonia* in mind. Schmitz (1883, p. 228) stated clearly that cystocarp could be applied to a gonimoblast with or without a wall and Svedelius has supported this view (1942b, p. 80). Other writers (Oltmanns, 1922, Vol. 2, p. 380; Kylin, 1930, p. 96; Fritsch, 1945, p. 616) reserve the term, however, for those types which possess a wall or pericarp derived from the gametophyte. Clearly a term is needed for the growth originating from the fertilized carpogonium and for that alone and of the two under consideration, carposporophyte seems preferable as apart from its possible associations with specific cases where pericarps are present, the meaning of the term cystocarp (a cavity containing sexually produced spores) is definitely unsuitable in others. Church's term *carposporophyte* has the merit of being self-explanatory to a certain extent and is suitable for use in a consideration of life-histories in that it is similar to gametophyte and tetrasporophyte. It seems best, however, to attempt a redefinition in order to avoid misunderstanding and confusion. *The carposporophyte of the Florideae is, therefore, that phase of development, initiated by fertilization and consists of only those growths, the origin of which can be traced to the fertilized carpogonium itself, i.e. the gonimoblasts.*

It seems essential also to consider the definition of the term *gonimoblast.* Zerlang (1889) attributes the first use of the term to Schmitz who, he says, defined it as the fertile tissue of a single cystocarp, formed by the outgrowths of a single fertilized initial cell, whether the fertilized egg cell or the fertilized auxiliary cell. Kylin (1937) also appears to have used it for the total of the spore-producing filaments, formed from a fertilized carpogonium or auxiliary cell, referring to the individual component filaments as gonimoblast filaments. On the other hand, Fritsch (1945, p. 599) and Smith (1944, p. 19) appear to consider each individual carpospore-producing filament formed from a fertilized carpogonium as a gonimoblast. The latter appears to be the appropriate use of the term but, at the same time, it seems desirable to enlarge this concept and to regard as a *gonimoblast, the sporangium-producing filament which develops from each initial outgrowth from either a fertilized carpogonium or generative auxiliary cell.*

Finally, it becomes necessary to consider a suitable term for the unified structure resulting from the correlated growth of the carposporophyte and surrounding " tissue " of the gametophyte. It might be claimed that cystocarp could be used but the strict meaning of the word makes it definitely unsuitable in some instances and in addition it has also been used in the same sense as carposporophyte. Hence the new term *gonimocarp* is suggested, and can be defined as *the structure found is some Florideae, consisting of the carposporophyte (i.e. all of the gonimoblasts) together with such " tissue " of the gametophyte, which surrounds the former and whose development is correlated with fertilization.*

The definition of *auxiliary cell* is left until a later section of the paper, for reasons which will then be apparent.

General Features of the Carposporophyte

The carposporophytes of the Florideae, whatever their organization, have several characteristics which will be referred to briefly. The first of these is mentioned by Church (1919), who states that the carposporophyte is prevailing diploid. While it is true that in the majority of cases investigated cytologically both the carposporophyte and the spores liberated from it are diploid, yet in others, reduction division takes place immediately after fertilization and so both the resulting carposporophyte and the spores to which it gives rise are haploid.

Church also mentions another characteristic, namely that the carposporophyte is parasitic on the gametophyte. In all but the simplest Florideae known today,

the carposporophyte is totally dependent on the gametophyte for its food supply and as a result of the germination of the zygote *in situ* anchorage is also provided. Thus, only the main function of spore formation remains, and a study of the organization of the carposporophyte must be considered in the light of these facts. Such elaboration and specialization, therefore, as has taken place would be expected to result in either the production of as great a number of spores per fertilization as possible or the best protection and nourishment of such spores as are formed. Indeed, in considering the various types of carposporophyte to be found in the Florideae, this is a possible interpretation of the intricate processes and characteristics noted. It should be stressed at the same time, however, that nothing is known of the physiology of the developing carposporophyte and investigations are much needed in this field. In all non-filamentous types the fertilized egg cell is to be found in the assimilatory layers of the gametophyte and often in those regions which are still in the process of active development.

In the course of development of a very simple carposporophyte such as that of *Batrachospermum*, it is seen that after fertilization, a small number of short filaments develop from the carpogonium. These filaments are all alike and bear carposporangia both terminally and laterally. Each carposporangium liberates a single spore. In some species, cells in and below the carpogonial branch give rise to involucral filaments.

A survey of the carposporophytes described in the literature shows that few are as simple as that of *Batrachospermum* but that among those showing a more elaborate structure, certain trends are recognizable. Some types may exemplify more than one of these trends. Excluding those features which are connected with correlated changes in the gametophyte, these are:

1. The fusion of the carpogonium and/or the gonimoblasts which develop from it, with *specified cells* of the gametophyte occupying definite positions.

2. The transfer of the nucleus resulting from fertilization or its derivative or derivatives to one or more *specified cells* of the gametophyte.

3. The distribution of spore formation resulting from one fertilization over a a large area of the gametophyte.

It must be emphasized that the fusions mentioned under the first heading are quite distinct from those which take place in a great variety of types at the base of well-developed and almost mature carposporophytes. A clear distinction should be made also between well-defined *cells occurring in specified position* with which the fertilized carpogonium or the developing gonimoblasts fuse early in the development of the carposporophyte on the one hand and *nutritive tissue* or *cells* with which no open cellular contact is established. The occurrence of secondary pit-connections late in the development of the carposporophyte between gonimoblasts and nutritive " tissue " is reported in some cases.

Types of Carposporophyte to be found in the Florideae

On the basis of the fundamental trends shown by the carposporophytes of the Florideae, it is possible to propose a classification of the known types. It cannot be considered in any way exhaustive however, as it is limited to those for which well-substantiated and relatively complete information exists. Conspicuous among the families, not represented, are the Endocladiaceae, Callymeniaceae, Gigartinaceae and Phyllophoraceae. The last-named family is of special interest in view of the fact that a tetrasporangium-producing nemathecium[3] replaces the carposporophyte in some species. The formation of tetrasporangia in place of carposporangia is also found in the carposporophytes of four species of *Liagora* and one species of *Helminthocladia*, it should be recalled.

In another group of families, including the Rissoellaceae, Mychodeaceae and Acrotylaceae, the available information suggests that one generative auxiliary cell is associated with several carpogonial

3. This term is in need of definition as it is used for various types of structure.

branches, but it is unknown whether more than one carpogonial branch functions.

Four major groups of types of carposporophyte can thus be recognized. Although the largest group it is admitted that Group D is a heterogeneous assemblage but the types included are alike in that the fertilization nucleus or its derivative is transferred to one or more cells of the gametophyte from which gonimoblasts arise. In the present imperfect state of our knowledge, particularly regarding the first post-fertilization events, it is impossible to do more than subdivide the group on the basis of the position of the generative auxiliary cell and its time of formation.

GROUP A — Gonimoblasts arising from the carpogonium; no fusion with cells of gametophyte before initiation of gonimoblasts; no transfer of fertilization nucleus or derivative to any cell of gametophyte; carposporophytes either compact or diffuse.

GROUP B — Gonimoblasts arising from the carpogonium; fusion of fertilized carpogonium with specified cell of gametophyte before their formation and/or fusion of gonimoblasts during development with specified cells of gametophyte at some distance from carpogonium; no transfer of fertilization nucleus or derivative to any cell of gametophyte; carposporophytes mostly diffuse.

GROUP C — Fertilization nucleus or derivative transferred by primary gonimoblasts to specified cells of gametophyte at some distance from carpogonium; secondary spore-producing gonimoblasts arising from these cells; carposporophytes diffuse.

GROUP D — Fertilization nucleus or derivative transferred to specified cell or cells of gametophyte relatively near carpogonium; gonimoblasts arising from these cells; carposporophytes compact, the majority showing a high degree of specialization.

1. Fertilization nucleus or derivatives transferred direct to one or more cells of carpogonial branch.

2. Fertilization nucleus or derivative transferred to supporting cell or to cell in sterile branch borne by supporting cell.

(a) Cell receiving fertilization nucleus or derivative, present before fertilization.

(b) Cell receiving fertilization nucleus or derivative, formed after fertilization.

Types belonging to these groups will now be considered briefly although certain examples, particularly those which have a bearing on the general thesis but are less well known, are selected for fuller mention.

GROUP A — Members of the Chantransiaceae and Batrachospermaceae belong to this group as well as some of the Helminthocladiaceae and Bonnemaisoniaceae. Many of these are so well known that fuller reference is unnecessary. In most of them the gonimoblasts are short, compactly arranged and show little differentiation as already described for *Batrachospermum*. In some of the Helminthocladiaceae which belong to this group, the carpogonium divides by a transverse wall after fertilization and the gonimoblasts develop from the upper cell only (*Nemalion*, *Liagora* and *Helminthora*). In others, fusions between the cells of the carpogonial branch beneath the developing carposporophyte proceed to degrees varying between species of the same genus, but such fusions always take place *after* the gonimoblasts are well developed.

Nutritive cells are associated with the carpogonial branch in some types (*Trichogloea*) and in other genera filaments which originate from the gametophyte grow round the developing gonimoblasts.

An exceptionally highly specialized type for this group is exemplified by *Bonnemaisonia asparagoides* (Svedelius, 1933). Here nutritive cells are formed by cells of the carpogonial branch, the gonimoblasts are differentiated into a lower sterile and an upper fertile region and a flask-shaped pericarp encloses the carposporophyte, resulting in a definite gonimocarp.

Another type of carposporophyte found in this group is illustrated by *Sirodotia* (Kylin, 1912; Skuja, 1931). This is a genus of freshwater species very like *Batrachospermum*, differing from it in the asymmetrical shape of the carpogonium. The gonimoblasts are few in number and grow in diverging directions but remain

parallel to the surface of the gametophyte. They may reach a considerable length and lateral branches develop from them on the side towards the surface of the gametophyte. The formation of sporangia is limited to these lateral branches. Similar carposporophytes are found in *Nothocladus* and *Cumagloia*.

Apart from *Bonnemaisonia asparagoides*, the types of carposporophyte included in this group are the simplest known. In many instances, they are immersed in the loosely constructed gametophytes, which show little, if any, correlated growth and there is little differentiation of the gonimoblasts. It is to be noted that a diffuse type of carposporophyte, in which the gonimoblasts grow between the filaments of the gametophyte, is found in two families, the other genera of which have compact carposporophytes.

Group B — Apart from the Naccariaceae and Gelidiaceae, the types grouped here are represented by single genera from various families of the Nemalionales, Cryptonemiales and Gigartinales (*sensu* Kylin, 1937), and are intermediate types which may have been much more common during one period of the evolutionary history of the Florideae and illustrate the first trend listed on p. 57. They are particularly instructive in the interpretation of the carposporophytes of Group C. Two subdivisions can be recognized, those showing fusions between the carpogonium and a specified cell of the gametophyte (usually a cell of the carpogonial branch) and those showing fusion between the gonimoblasts and specified cells of the gametophyte at some distance from the carpogonium. In some instances fusion of the carpogonium with neighbouring cells may occur as well. In the former subdivision are *Dermonema* (Helminthocladiacae), *Naccaria* and *Atractophora* (Naccariaceae). *Bertholdia* (Calosiphoniaceae), *Nemastoma* (Nemastomaceae), *Cruoria* (Cruoriaceae), *Polyides* (Rhizophyllidaceae) and *Acrosymphyton* (Dumontiaceae) demonstrate the fusion of gonimoblasts with specified cells of the gametophyte. The types of this group appear to be of particular interest and some of the examples will now be considered in greater detail.

In *Dermonema* (Svedelius, 1939) the carpogonium fuses with the supporting cell of the carpogonial branch before the formation of the gonimoblasts. The latter resemble those of *Sirodotia* in that they spread between the filaments of the gametophyte remaining more or less parallel to its surface. Also like *Sirodotia*, they give rise to lateral spore-producing branches at right angles to the creeping filaments, thus showing some differentiation. No nutritive and protective cells are produced by the gametophyte. *Naccaria* and *Atractophora* (Kylin, 1928) resemble *Dermonema* in that the carpogonium fuses with a specified cell of the gametophyte (the hypogynous cell in the case of *Naccaria* and the supporting cell of the carpogonial branch in the case of *Atractophora*) before giving rise to the gonimoblasts which from a carposporophyte of the diffuse type. In addition, branches of the carpogonial branch form a special nutritive tissue and fertilization induces some further growth of the filaments of the gametophyte in the neighbourhood of the developing carposporophytes. The carposporophytes of the Naccariaceae thus show a higher degree of elaboration.

Although not showing any very close relationship with the types under discussion, *Gelidium*[4] is relevant in that it appears to be another intermediate type between those of Groups A and B. The carpogonium enlarges after fertilization and may fuse with either the hypogynous cell or other neighbouring cells before further development takes place. Such fusions are not obligatory, however, and the cells with which the carpogonium may fuse do not appear to be specifically determined. Long processes arise from either the carpogonium or the fusion cell, from the tips of which the gonimoblasts develop. These are differentiated into filaments growing parallel to the long axis of the branch in which the carpogonia develop, and short spore-producing filaments at right angles to the former. Both nutritive and protective "tissues" are formed by the gametophyte.

Among the types which show fusion of the gonimoblasts with specified cells

4. I am indebted to Dr. P. S. Dixon for permission to make use of unpublished data.

of the gametophyte at a distance from the carpogonium, *Bertholdia* probably represents the most primitive. Feldmann (1952a) has shown that after fusion of the fertilized carpogonium with cells of the carpogonial branch, four gonimoblasts arise from the fusion cell. They grow and branch among the cells of the gametophyte, parallel to its surface and fuse, with certain intercalary cells of the gametophyte filaments. Short branches arise at intervals on, and at right angles to, the main gonimoblasts and give rise to the spores, the groups of spores being greater in number than the fusions. No nucleus from the gonimoblasts is transferred to the cells of the gametophyte with which they fuse. The similarity between this carposporophyte and that of *Dermonema* to the type of Group A, as illustrated by *Sirodotia* on the one hand and that of the closely related *Calosiphonia* of Group C on the other, provides a helpful clue to the origin of Group C. It suggests that after the occurrence of fusion between spreading gonimoblasts and certain specified cells of the gametophyte at some distance from the carpogonium, progressive localization of the lateral spore-producing branches to the neighbourhood of the fusions has taken place. In *Cruoria*, for example, the spore-producing branches are not very near to these cells, but in *Nemastoma* they develop comparatively near the fusion cell. Finally, in *Acrosymphyton*, the spore-producing laterals develop from the gonimoblast itself as in the other cases, but right at the point of fusion with the cell of the gametophyte. It is to be noted that tetrasporophytes are unknown for many of these genera. The carposporophytes are immersed in the loose cortical regions of the gametophyte from which no special nutritive tissue arises, and consist, as has been shown, of gonimoblasts, differentiated into creeping main axes and short, spore-producing lateral branches growing at right angles to the former.

The further elaboration of the types shown by *Bertholdia*, *Nemastoma* and *Acrosymphyton*, by the transfer of a derivative of the fertilization nucleus to specified cells of the gametophyte with which the gonimoblasts fuse and the development of the spore-producing laterals from those cells, is considered under the next heading.

Group C — The types included here all belong to the Cryptonemiales as delimited by Schmitz and Hauptfleisch (1897), a group of families Kylin (1937) divided between his Cryptonemiales and Gigartinales. Kylin included in the former, forms in which the auxiliary cells are situated in special accessory branches. Genera belonging to the Gloiosiphoniaceae, Dumontiaceae and Grateloupiaceae of Kylin's Cryptonemiales are considered here and also others belonging to the Nemastomaceae, Furcellariaceae, Solieriaceae and Rhabdoniaceae of Kylin's Gigartinales.

This type, as represented by *Dudresnaya coccinea*, has been well known since the time of Oltmann's researches. The carposporophytes of this group represent the most specialized of those in which spore formation resulting from a single fertilization is distributed over a large area of the gametophyte. In addition, together with those of Group D, they show the most general feature of the carposporophytes of the Florideae, namely the transfer of derivatives of the fusion nucleus to specified cells of the gametophyte, cells generally referred to as *auxiliary cells*. In this group, this transfer is effected by the gonimoblasts. As in certain types of Group B, the gonimoblasts are few and long and the auxiliary cells are at a considerable distance from the carpogonium. After each fusion between a gonimoblast and an auxiliary cell, a nucleus passes from the former into the latter, from each of which, one or more compact spore-forming branch systems then develop. The carposporophyte is, therefore, spread throughout the cortical assimilatory layers of the gametophyte and consists of a few long primary gonimoblasts, which fuse with auxiliary cells and small groups of secondary short compact branch systems, bearing sporangia.

Like *Dudresnaya coccinea* many of the forms here included show no further elaboration. The primary gonimoblasts may arise from the carpogonium without prior fusion between it and other cells of the carpogonial branch (*Grateloupia*,

Platoma) or after such fusion (*Dumontia*). In *Pradeaea* (Feldmann, 1942) special nutritive cells are formed from both the cell above and the cell beneath the auxiliary cell and in *Titanophora* (Feldmann, 1942) protective tissue develops from the auxiliary cell.

Furcellaria differs from the simpler forms in that the carpogonial branches are localized in the tips of the branch system and so it is likely that several carposporophytes are intermingled.

In the Solieriaceae and Rhabdoniaceae are found more specialized and elaborate types in which there is correlated development shown by the gametophyte around the individual spore-producing branch systems, formed from each auxiliary cell. In *Agardhiella tenera* (Kylin, 1928), for instance, two or three gonimoblasts grow from the fertilized carpogonium, and the cells with which they fuse are few in number and have rich contents as have also those cells with which they are in pit-connection. The single secondary gonimoblast forms a sphere of pseudoparenchymatous "tissue", certain cells of which establish contact with cells of the surrounding nutritive "tissue" formed after fertilization by cells of the gametophyte. Finally more filamentous branches spread out from the pseudoparenchymatous sphere and bear the carposporangia, the cells of the gametophyte meanwhile dividing to form a protective layer in which there is a definite pore, through which the spores escape.

In some of the more elaborate types, especially those of the Rhabdoniaceae, a large fusion cell, derived from the basal cells of the gonimoblasts and the auxiliary cell, is present at the base of the mature carposporophyte.

The interpretation of these carposporophytes is postponed until the general discussion. In this group, the dispersal of spore formation from a single fertilization reaches a high degree of elaboration. While the secondary branch systems producing the spores show little differentiation in many types, yet a few reach a considerable degree of differentiation. In these more elaborate types, correlated growth of the gametophyte is found around each such group of spores, comparable to that found around the whole carposporophyte in other groups.

Group D — It cannot be claimed that this group is at all well understood at the present time. The types included here are all alike in that the fertilization nucleus or a derivative is transferred to one or more auxiliary cells (i.e. specified cells of the gametophyte) situated near the carpogonium. The carposporophytes, therefore, are compact structures consisting in most instances of a single group of spore-producing filaments.

Sub-group D/I — In Group B certain types were described, in which the carpogonium fuses with a cell of the carpogonial branch or the supporting cell, before giving rise to the gonimoblasts and it would seem a small step from such a state of affairs to one where the fertilization nucleus moves over to this other cell which then gives rise to the gonimoblasts. The close relationships between more primitive types and those of this group is shown by the Bonnemaisoniaceae. The gonimoblasts of *Bonnemaisonia* develop from the carpogonium, but in the nearly related *Asparagopsis armata* (Svedelius, 1933) the fertilization nucleus enters the hypogynous cell which then gives rise to two or more gonimoblasts. These together form a pseudoparenchymatous "tissue" from the outer surface of which both sterile paraphyses as well as carposporangia-producing filaments arise later. Special nutritive tissue and a flask-shaped protective covering formed by the gametophyte are present in this comparatively highly specialized type of carposporophyte.

Two genera of the Chaetangiaceae should be mentioned here, *Scinaia furcellata* (Svedelius, 1915) in which the branched but undifferentiated gonimoblast develops from one of the four hypogynous cells and *Galaxaura* (Svedelius, 1942a) which shows an elaboration of a carposporophyte of quite a different type. Here the diploid nucleus or its derivative passes into the hypogynous cell, but then further daughter nuclei migrate to other cells of the branched carpogonial branch. Gonimoblasts develop from various cells of the carpogonial branch, some of them growing to a considerable length before

branching and forming carposporangia. This accounts for the occurrence of secondary groups of spores around the central one, in the mature structure. As the carposporophyte develops, fusion of the cells of the carpogonial branch takes place.

While some of these carposporophytes are very highly specialized, yet it would appear that they have been derived from the *Batrachospermum* type either directly or through forms where there is fusion between the carpogonium and another cell of the carpogonial branch without any transfer of the fusion nucleus.

It will be noticed that the types of this sub-group occur in the two highly developed families of the Nemalionales (*sensu* Kylin), the Bonnemaisoniaceae and the Chaetangiaceae. Kylin considered that the cells from which the gonimoblasts arise in these examples are not true auxiliary cells. This is referred to again in the discussion.

Sub-group D/II — Whereas the fertilization nucleus or its derivative is transferred to a cell or cells in close association with the carpogonium in the previous sub-group, the association between carpogonium and auxiliary cell is not so close here. Unfortunately the information regarding the method of transfer of the nucleus is very scanty and so the relationships of the various types is not clear. Two subdivisions are apparent nevertheless, the one consisting of the Ceramiales, in which the auxiliary cell is formed after fertilization and the other consisting of families included by Kylin in his Gigartinales (Hypnaceae, Rhodophyllidaceae, Gracilariaceae, Sphaerococcaceae and Plocamiaceae), Rhodymeniales and Crytonemiales [Gloiosiphoniaceae (in part only) and the Corallinaceae]. Many belonging to this second group (Sub-group D/II/a) as well as possibly Gigartinales, for which the details are not available, show some of the most highly specialized carposporophytes to be found in the Florideae. For the sake of brevity only a limited number of examples illustrating different types can be referred to.

Sub-group D/II/a — One of these is *Calliblepharis lanceolata* (Kylin, 1928) in which the generative auxiliary cell is the basal cell of a lateral branch of the supporting cell. The method of contact between the carpogonium and this cell is unknown, but a single gonimoblast is cut off to the inside of this cell. At the same time a number of the surrounding cells of the gametophyte give rise to small cells with dense contents and the outer cortical cells initiate the development of the pericarp. While in the early stages, the food supply of the developing carposporophyte comes from the cells in pit-connection with the generative auxiliary cell, later on the developing gonimoblast enters into secondary pit-connection with the small-celled nutritive tissue which surrounds it. Later unbranched filaments develop from the pseudoparenchymatous cushion and give rise to carposporangia. As the carpogonial branches are formed in special small branches of the thallus, the mature carposporophytes are to be found in a fringe of small capsule-like bodies. It is obvious that in this case the carposporophyte reaches quite a high degree of specialization.

Gracilaria multipartita also reaches a high degree of specialization but of a different type, and I am indebted to Mrs. E. Greig-Smith for permission to use unpublished results of an investigation she has carried out. In addition to the carpogonial branch, the supporting cell bears three other short branches. One of the latter contains the auxiliary cell, while the other two develop into normal cortical branch-systems. After fertilization, a short filament connects the carpogonium with the generative auxiliary cell, which after the entry of the diploid nucleus enlarges. A transverse wall separates it into a small inner or lower cell and a large upper containing the diploid nucleus. This cell then fuses with a number of vegetative cells, the diploid nucleus dividing several times meanwhile and ultimately a very large fusion cell-results. This fusion-cell is not to be compared with those to be found at a later stage in the development of, and at the base of, mature carposporophytes, but has more points in common with the fusion cell sometimes present in the carposporophyte of *Gelidium*. Such a cell contains at a certain stage both haploid and di-

ploid nuclei, the latter taking part in the formation of gonimoblasts from the periphery of the fusion-cell. At first the gonimoblasts are in lateral contact and give rise to a pseudoparenchymatous "tissue" from the surface of which sporangium-producing filaments develop later. In addition, haustorial-like filaments develop from both the pericarp, formed from neighbouring cortical filaments of the gametophyte, and also from the pseudoparenchymatous central tissue of the carposporophyte adding, no doubt, thereby, to the food supply of the developing carposporophyte. There are thus certain resemblances in the early stages between what has just been described for *Gracilaria* and what is known for the Rhodymeniales, the main difference being the fusion of the auxiliary cell with neighbouring cells of the gametophyte before the initiation of the gonimoblasts. It should be noted that the details of the early stages in the development of the carposporophyte differ from those given by Sjöstedt (1926) for *Gracilaria* spp. and by Papenfuss (1935) for the related *Melanthalia abscissa*.

In other cases in which the carpogonium and the generative auxiliary cell occur in the same fertile branch system, the supporting cell appears to act as the generative auxiliary cell and the most clearly investigated case is that of *Sphaerococcus coronopifolius* (Sjöstedt, 1926). The method by which the diploid nucleus is transferred from the carpogonium to the supporting cell is unknown, but after fertilization the contents of not only the supporting cell but also those of the neighbouring cells become denser. This process continues after the formation of the first gonimoblast and extensive fusions have taken place. In all, several gonimoblasts which remain distinct develop from the generative auxiliary cell.

The carposporophyte of the Corallinaceae has been variously interpreted. Here several fertile branches develop side by side in the base of a conceptacle and each has been interpreted as consisting of a basal auxiliary cell with or without an apical rudiment and bearing one or sometimes two 2-celled carpogonial branches. Thus the auxiliary cell is the supporting cell. There is some doubt about the immediate post-fertilization details, but in one instance (*Melobesia Lejolisii*) Suneson (1937) observed a short outgrowth from the carpogonium which fused with the corresponding auxiliary cell. Minder (1910), on the other hand, saw the carpogonium of *Choreonema Thureti* fuse directly with the auxiliary cell beneath, but no movement of the diploid nucleus took place. Whatever the details of this early stage however, the auxiliary cell which fuses with, or is connected to a fertilized carpogonium fuses laterally with all the other supporting cells of the conceptacle to form a large fusion-cell in which both haploid and diploid nuclei occur. The short sporangium-forming gonimoblasts develop usually from the periphery of this cell, but sometimes from the central region also. It is not clear whether more than one fertilization may be effective within a single conceptacle.

The carposporophytes referred to here probably represent different evolutionary lines. Our knowledge of them and nearly related forms is so incomplete that a general statement would lack validity. It is apparent, however, that all these carposporophytes are highly specialized.

Sub-group D/II/b — This comprises the well-known order Ceramiales, of which a number of examples have been investigated, the best known being *Polysiphonia*. Throughout the order, the carposporophyte develops from a cell cut off from the supporting cell after fertilization. In some genera of the Ceramiaceae a second auxiliary cell is associated with a single carpogonium, but is to be interpreted as the remains of a second carpogonial branch of a specialized fertile axis. In such cases, two groups of gonimoblasts are formed in close proximity. The four families of the Ceramiales show various elaborations of this type of carposporophyte.

Plumaria elegans (Drew, 1939) may be referred to as an example of the type found in the Ceramiaceae. Here the carpogonium cuts off a small cell on the side towards the elongated supporting cell. The latter divides by a transverse wall into a larger outer cell, the generative

auxiliary cell and a smaller inner cell. Open contact is established between the auxiliary cell and the small cell cut off by the carpogonium following which the diploid nucleus of the latter enters the auxiliary cell. The haploid nucleus of the auxiliary cell is isolated from it by the formation of a wall near and parallel to the wall by which the auxiliary cell was formed. The cell containing the diploid nucleus, known as the central cell, gives rise to three, four or five gonimoblasts. Filaments growing from nearby cells envelop the carposporophyte.

In *Polysiphonia* (Rhodomelaceae) groups of sterile cells are associated with the fertile branch and probably have a nutritive function. After fertilization, the fusion nucleus divides and the auxiliary cell is cut off from the supporting cell. The carpogonium then fuses with the auxiliary cell and one of the diploid nuclei passes through into the latter. Kylin (1923) reports that the diploid nucleus then gives rise to a cell from which the sympodially branched gonimoblast develops. Even before the entry of the diploid nucleus, the auxiliary cell begins to fuse with the supporting cell and this process then extends to the central cell of the fertile axis. The small carposporophyte is protected by a flask-shaped pericarp formed from neighbouring cells of the gametophyte.

The carposporophytes of the Delesseriaceae are essentially similar, but are immersed in the thalli, sometimes in special branchlets, so that the mature gonimocarps have the appearance of capsules. Fertilization stimulates the formation of a pericarp from cells of the gametophyte in these and other forms. Fusion-cells resulting from the older cells of the gonimoblasts, the auxiliary cell, the supporting cell and connected cells of the gametophyte are found at the base of the mature carposporophytes.

While their connection with the simpler types is not clear, the formation of the auxiliary cells after fertilization is so constant a feature throughout the Ceramiales that it seems likely that they represent a line of evolution long distinct.

Discussion and Conclusions

In this paper it has been suggested that the most primitive type of carposporophyte known consists of a number of undifferentiated gonimoblasts which arise from the carpogonium direct, such as is seen in *Batrachospermum*, and that among other carposporophytes so far investigated, three major trends in specialization can be recognized (p. 57). On the basis of these trends, types for which well-documented information exists have been classified into four groups, the main features of each group being given on p. 58. Various types to be found in each group have been considered in further detail.

As a result of this comparative review, two major conclusions seem warranted. Firstly, the transfer of the fertilization nucleus, such as is seen in Group C and possibly certain members of Group D, has arisen from the condition in which specified cells of the gametophyte fuse with the carpogonium or the gonimoblast, without any accompanying transfer of a nucleus, a condition exemplified by the types of Group B. Secondly, two main lines of specialization can be recognized:

(1) The dispersal of spore-formation resulting from one fertilization by means of long diverging gonimoblasts, and

(2) the elaboration and differentiation of a few gonimoblasts, sometimes one only, usually formed from an auxiliary cell near the carpogonium, the method of transfer being so far incompletely understood.

Transfer of the Fertilization Nucleus — The transfer of the fertilization nucleus or its derivative occurs in the vast majority of existing Florideae. If the examples quoted in Group B are considered however, that is those types where fusion with specified cells of the gametophyte occurs without any transfer of a nucleus, it is found that they are closely allied to types in both Groups A, C and D/I. *Dermonema*, for example, is a member of the Helminthocladiaceae, the majority of which belongs to Group A. The Naccariaceae which belong to Group B and which show fusion of the carpogonium with certain cells of the carpogonial branch before the

formation of the gonimoblasts, are closely allied to the Bonnemaisoniaceae, one genus of which *Bonnemaisonia* is in Group A and another *Asparagopsis* is in Group D. These examples concern fusion of the carpogonium and cells of the gametophyte, but even more pertinent are those which concern the fusion of the gonimoblasts with specified cells of the gametophyte at some distance from the carpogonium. If the pairs of closely related genera *Bertholdia-Calosiphonia* and *Acrosymphyton-Dudresnaya* are compared, it is found that in the first mentioned of each pair the spore-producing branch arises from the primary gonimoblast (at the point of fusion in *Acrosymphyton*, but at any point in *Bertholdia*), but in the second, it arises from the cell of the gametophyte, after entry of a derivative of the fusion nucleus, contributed by the gonimoblast after fusion. It seems clear from these examples that the specified cells of the gametophyte, with which the gonimoblasts fuse, are homologous in all these genera and that the transfer of a derivative of the fertilization nucleus to such a cell is a secondary condition. Feldmann (1952a) has already pointed out the homology of the cells in question in the case of *Bertholdia* and *Calosiphonia* and suggested that the term *auxiliary* cell should be defined to include both cases. This seems logical, but it would also seem desirable to include all the types of Group B. Indeed the term was first used by Schmitz (1883, p. 229) for this particular cell of *Naccaria*. Later the term was redefined by Kylin (1923) who considered a *typical* auxiliary cell to be a cell, other than the carpogonium or a cell of the carpogonial branch, from which gonimoblasts (*sensu* Kylin) arise. Kylin gives no reason for this exclusion of the cells of the carpogonial branch, but it probably rests on the fact that the nucleus is not transferred by a connecting filament (to use Kylin's terminology) in such cases. While following Martin (1939) and Svedelius (1942b) in disagreeing with Kylin on this particular point, students of the Florideae have not seriously questioned Kylin's definition of typical auxiliary cell. Papenfuss (1951) has suggested that a distinction should be made between *nutritive* and *generative auxiliary cells*, but appears to include a number of different types of cell under the first term, while reserving the second for those to which the fertilization nucleus or its derivative is transferred and which then give rise to spore-producing gonimoblasts. A distinction must be made, however, between genuine *nutritive auxiliary cells*, i.e. specified cells with which the carpogonium or gonimoblasts fuse either, in the case of the carpogonium, before the formation of the gonimoblasts or, in the case of the gonimoblasts, before the formation of the spore-producing filaments, and *nutritive cells* and *nutritive tissue*, with which no fusion takes place. The following definition is, therefore, proposed:

An auxiliary cell is a specified cell of the gametophyte with which the carpogonium fuses before the formation of gonimoblasts or a cell with which a primary gonimoblast fuses. Auxiliary cells have a purely nutritive function (nutritive auxiliary cell) in those cases where no nucleus is transferred, or combine nutritive and generative functions (generative auxiliary cell) in those cases where the fertilization nucleus or its derivative is transferred to that cell and there initiates the development of secondary gonimoblasts.

On the basis of this definition, auxiliary cells are absent in types of carposporophyte of Group A, nutritive auxiliary cells are present in those of Group B. While generative auxiliary cells are present in all types of Groups C and D, nutritive auxiliary cells are sometimes present in addition in Group C.

Main Lines of Specialization of the Carposporophyte — Types with long diverging gonimoblasts, resulting in the spread of spore formation from a single fertilization over a considerable area of the gametophyte, have been described in Groups A and B, e.g. *Sirodotia*, *Dermonema* and *Bertholdia*. It has been shown how by further localization of the spore-producing laterals, first to the place of fusion with the nutritive auxiliary cells and then to the generative auxiliary cells themselves, the type of carposporophyte of Group C may have arisen. The carposporophyte of *Dudresnaya* and others of Group C consists, therefore, of a number

of sterile gonimoblasts which fuse with the generative auxiliary cells, from which spore-producing laterals arise. In this type of carposporophyte, a distinction has been made usually and different terms used for the filaments connecting the carpogonium and the auxiliary cell and the spore-producing filaments which then arise from the generative auxiliary cell. The latter have been referred to as gonimoblasts or gonimoblast filaments and the former as either "ooblastema" (Schmitz, 1883), "verbindungsfaden" (Berthold, 1884) or sporogenous filaments (Oltmanns, 1898). Oltmanns, it should be noted, used the same term for the gonimoblasts of the Nemalionales. In consequence of the interpretation of these carposporophytes now put forward, the filaments connecting the carpogonium and the generative auxiliary cells are to be regarded as gonimoblasts and the spore-producing branch systems as evolved from the lateral branches of these gonimoblasts. It would seem best to recognize this by referring to the former as *primary* and the latter as *secondary* gonimoblasts.

In Group C increasing differentiation of the secondary gonimoblasts is seen and the term *gonimolobe* might be suitably applied to each individual group of secondary gonimoblasts. In some more specialized types, there is correlated development of gametophyte "tissue" around each gonimolobe such as in *Agardhiella*, resulting in a unit resembling a gonimocarp. Since each such unit is only part of the carposporophyte, it might be more suitably referred to as a *microgonimocarp*.

The second main line of specialization appears to have been that of elaboration and specialization of a few and, in many cases, one gonimoblast only from a single generative auxiliary cell placed relatively near the carpogonium. In one group of such carposporophytes, those placed in Group D/I, the generative auxiliary cell is in the carpogonial branch and the transfer of the fertilization nucleus or its derivative takes place by direct migration. It is likely that this condition has arisen from the types of Group A, either directly or through the intermediate condition of Group B, for in the Bonnemaisoniaceae, for example, the gonimoblasts develop from the carpogonium in *Bonnemaisonia*, but from the hypogynous cell in the closely allied *Asparagopsis*. The high degree of differentiation reached by this carposporophyte has been referred to already.

The origin and relationships of the remaining types of carposporophyte found in this group (Group D/II) are obscure. This is due in part to the absence of intermediate types but also to the lack of information about the method of transfer of the fertilization nucleus or its derivative to the generative auxiliary cell, particularly in types other than those of the Ceramiales. In that order as well as in others, the available information suggests that this is effected sometimes by direct fusion between the carpogonium and the generative auxiliary cell sometimes by means of a thin tube and sometimes by means of an intermediate cell, formed by the carpogonium. Until the significance of this is understood, it is not possible to discuss whether the gonimoblasts from the generative auxiliary cells are in fact primary or secondary. Although their relationship to other orders is obscure, the Ceramiales undoubtedly represent a well-defined group and probably a distinct line of development. This is borne out not only by progressive specialization of the gametophyte but also by the carposporophyte, which shows a range of increasing complexity from the comparatively simple type found in the Ceramiaceae. It is interesting to note, however, that in certain Ceramiaceae (*Spermothamnion*, for example) the product of a single fertilization nucleus is transferred to two generative auxiliary cells and so the situation is analogous to, if not homologous with, that found in Group C.

There is no doubt that the remainder of the types classed here in Group D/II and belonging to Kylin's Gigartinales, Rhodymeniales and Cryptonemiales represents advanced and specialized types. A certain degree of differentiation of the gonimoblasts is recognizable. In many the inner cells remain sterile and may give rise not only to spore-producing filaments but to sterile filaments in addi-

tion, which establish contact with the cells of the pericarp which are full of food materials as a result of photosynthetic activity. This has been described for *Gracilaria multipartita*, but is found in other genera such as *Hypnea* and *Rhodophyllis* also. In many of the types of this group, a large fusion-cell is present at the base of the mature carposporophyte, particularly in the two families of the Rhodymeniales, but is also found in *Cystoclonium* and *Rhodophyllis*, amongst others. This fusion-cell consists usually of the lower cells of the gonimoblasts, the auxiliary cell and sometimes neighbouring cells of the gametophyte. Although resembling the fusion cells in *Gracilaria* and *Corallina*, they are formed well after the initiation of the gonimoblasts instead of before them as in the case of the two mentioned. In the majority of these forms fertilization initiates the development of both nutritive tissue and a pericarp, the latter in many cases with a well-defined ostiole. The *Corallinaceae* stand rather apart from the other families included in this group but, there is no doubt that this carposporophyte is of a highly specialized type. The pericarp is formed well before fertilization and serves to protect the developing procarps as well as the carposporophyte. No special nutritive " tissue " is formed by the gametophyte.

In many of the genera belonging to this Group D/II, the procarps are formed in special branches of the thallus and hence the carposporophytes are localized and occupy definite positions on the thallus. The development of a pericarp, sometimes with an ostiole, results in the resemblance of the mature structure, the gonimocarp, to a capsule.

It has been shown that this type of highly specialized carposporophyte is to be found in various systematic orders, the Nemalionales, Gigartinales, Rhodymeniales and Ceramiales. It is to be doubted whether as many spores are formed per fertilization in carposporophytes of this type as in those of Group C, but as the vegetative structure of the gametophyte is correspondingly specialized, the conclusion that these types are highly evolved seems justified.

Classification — The bearing of the conclusions reached in this comparative review on the problems of the classification of the Florideae will be considered briefly. While recognizing that many characters in all phases of the life-history must be taken into consideration in determining the limits of an order, details of the carposporophyte are certainly of great importance in the classification of the Florideae. If the carposporophyte is correctly interpreted, it is to be expected that any major divisions based on such, will show a number of other features in common. While no definite proposals will be put forward at this stage, a number of suggestions for consideration by investigators of these algae are made. The observations are made in relation to the current system of classification (see Kylin, 1937).

Firstly, there are two groups of families in the Nemalionales, the one in which the carposporophytes are either comparatively or extremely simple and the other in which they are more elaborate or even highly specialized. The second group includes the three families, the Naccariaceae, Bonnemaisoniaceae and the Chaetangiaceae, and in the second and third of these families the gametophytes also are highly specialized. In addition the life-history of certain genera of the Bonnemaisoniaceae is complex as has been shown by Feldmann and Feldmann (1942) and is not yet understood. On the grounds of the life-history, these workers have suggested that the family be removed to a separate order, the Bonnemaisonales (loc. cit. p. 163). However, Levring (1953) has described tetrasporangia on individuals similar to the gametophytes for two species belonging to separate genera, *Leptophyllis conferta* and *Delisea pulchra*, genera which he considers typical members of the Bonnemaisoniaceae, and so it appears that the life-history varies in the family as it does in the Chaetangiaceae also. Caution appears to be necessary in delimiting fresh orders until further more extensive investigations have been carried out. Meanwhile the differences between these families and the others of the Nemalionales can be noted and it is also clear that the remaining families

comprise a more or less homogeneous group.[5]

The relationships of the family Gelidiaceae are not clear, but while showing affinity with the Nemalionales in the simple carpogonial branch it shows an advance on the simpler genera of that group in the elaboration of both gametophyte and carposporophyte.

Secondly, the separation of the Ceramiales by Oltmanns (1898) and maintained by Kylin (1937) has been abundantly justified and this remains the most clearly defined order of the Florideae. The high number of species in this order suggests that this is a highly successful type.

Thirdly, it would appear that with the possible exclusion of the Corallinaceae, the Cryptonemiales of Schmitz and Hauptfleisch (1897) is based on a fundamentally distinct type of carposporophyte. Even if further work shows that a further subdivision is necessary, the association of any of these families with others of the Gigartinales, as is done by Kylin, is not desirable as they represent basically different types of carposporophyte.

The *fourth* point which emerges is that no fundamental differences exist between certain families of the Gigartinales, such as the Rhodophyllidaceae, Hypnaceae and Gracilariaceae and those of the Rhodymeniales. However, until other families of the Gigartinales, which may represent quite a different line of development, the Gigartinaceae and the Phyllophoraceae in particular, have been thoroughly investigated, it would be premature to re-group the families of these two orders, the Gigartinales and Rhodymeniales. Likewise there are certain families of the Cryptonemiales of Kylin, such as the Callymeniaceae and the Endocladiaceae, whose systematic position will have to remain uncertain until thorough investigation of the carposporophytes has been undertaken.

While definite proposals regarding alterations in the classification of the Florideae will have to be based on investiagtions undertaken with that object in view, this review has served to point the need for such investigations as well as other investigations of the little known or completely unknown types of carposporophytes, which undoubtedly exist in addition to those referred to in this paper.

5. Feldmann (1952b) has proposed that the Acrochaetiaceae be given ordinal rank, on the basis of the extremely simple carpogonial branch.

Summary

The available literature on the development of carposporophytes of the Florideae has been surveyed and it is clear that our knowledge of the origin and development of this phase of the life-history is very imperfect in many families, some of the families of obvious importance.

In the most primitive type of carposporophyte a number of undifferentiated gonimoblasts arise from the carpogonium direct and there are no fusions with specified cells of the gametophyte. A comparison of the organization of other types shows that it is possible to recognize three trends: (1) fusion between specified cells of the gametophyte with either the carpogonium or the gonimoblasts, (2) transfer of the fusion nucleus or its derivative to specified cells of the gametophyte, and (3) the distribution of spore formation from a single fertilization over a large area of the gametophyte. On the basis of these trends a classification of the *known* types has been proposed and types belonging to the groups, thus delimited, are considered.

Two conclusions seem to be supported by this review of the organization of various types of carposporophytes. Firstly, that the transfer of the derivatives of a single fertilization nucleus to several generative auxiliary cells at some distance from the carpogonium has arisen from the condition in which gonimoblasts fuse with specified cells of the gametophyte during their development. Secondly, that two main lines of specialization of the carposporophyte can be recognized, the one showing the dispersal of spore formation by means of long gonimoblasts and the other the elaboration and differentiation of a few (sometimes one only) gonimoblasts.

Certain terms, such as *gonimoblasts*, *carposporophyte* and *auxiliary cell*, have been redefined and a new term *gonimo-*

carp is suggested for the unified structure consisting of the carposporophyte and surrounding " tissue " of the gametophyte.

As a result of this comparative review, it is suggested that Kylin's classification of the Florideae needs reconsideration. While suggestions are made, definite changes should be based on further investigations undertaken with this object in view.

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ORGANIZATION OF THE SHOOT APEX IN DICOTYLEDONS

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The organization of the apical meristem in dicotyledons has been described, in recent years, in terms either of the arrangement of cell divisions or of the appearance of the protoplasts. The former approach has been adopted by many authors and is now well understood. The concept of a tunica enclosing a corpus, on which it is based, is now established in modified form, and the constancy of these zones or their variation from species to species or during the course of development has been investigated at least adequately. The second approach, which postulates zones of cells dividing at different rates and with protoplasmic contents in different physiological states, has been less adequately pursued, and its general validity has been questioned. Since a former review of the subject (Philipson, 1948) a number of important investigations have been reported, and their implications will now be discussed.

It has never been suggested that the two methods of description are conflicting, but rather that they are concerned with different processes. It is possible that they can be unified, and the two aspects included in one concept of the organization of the apex. In very general terms it may be said that the dicotyledonous apex normally consists of (i) a central zone with infrequent divisions and protoplasts which stain relatively lightly, and (ii) a peripheral zone with more frequent cell divisions, resulting in smaller cells, which have more densely staining protoplasts. In both of these zones the cells at, or near, the surface normally divide anticlinally; in addition, the peripheral zone is intimately connected with the initiation of leaf primordia. The cells immediately below these zones form a transitional zone to the more mature tissue, which, if sufficiently distinct, forms a third meristematic zone, the rib-meristem. This third zone is intimately connected with the development of nodes and internodes. It is by division of the central zone that the other two meristematic zones are maintained.

The occurrence of such a zonation in dicotyledons has been confirmed by a number of authors. Although Gifford (1950) found that the cyto-histological zonation pattern of the stem apex was sufficiently distinct in several woody Ranales for some genera to be distinguished by this means, he described for all a central and a peripheral zone together with a rib-meristem developed to varying degrees. It is true that his descriptions confine these zones to the corpus and that he describes the tunica as a separate entity, but his excellent photomicrographs confirm that the zones extend to the surface of the apex and, therefore, include the tunica as well as the corpus. As my own descriptions have lacked clarity on this point, and consequently have been misinterpreted (for example, by Millington & Gunckel, 1950), I should record now that I consider that the central and peripheral zones include tunica cells as well as those of the corpus.

Steinberg (1950) compares the zonation of the apex of *Solanum lycopersicum* with that of *Succisa* (Philipson, 1947a). However, his zone 1 corresponds to the greater part of the apex and would include both the central and peripheral zones of *Succisa*. Whether this zone 1 of the tomato could be resolved into distinct zones cannot be determined from the figure published in Steinberg's paper, but unpublished work on the potato by one of my students (Fitzgerald, thesis for M.Sc.) shows the presence of central and peripheral zones in the apex of that species of *Solanum*.

In a paper concerned for the most part with the origin of the primary tissues in *Dianthera americana*, Sterling (1949) describes the zonation of the stem apex in terms reminiscent of those used by Grégoire (1938) in describing the apex of the flower. The apex of *Dianthera* comprises a core of lighter-staining cells with a mantle of denser cells. From an examination of Sterling's figures it would seem that the central core consists of maturing cells, corresponding to a rib-meristem, while the mantle comprises the more strictly meristematic part of the apex. That this mantle may be divisible into a central zone and a peripheral zone is suggested by Sterling's Fig. 18, where the cells to the flanks of the domed apex are distinctly smaller than those at the centre.

Millington and Gunckel (1950) describe central and flanking zones, and a rib-meristem in *Liriodendron tulipifera*. Their diagram indicates that they consider the central zone to include the tunica layers, as well as the corpus. They found no seasonal or plastochronic changes in organization.

Some authors have described alterations in organization during the ontogeny of the plant. In an investigation covering the life of *Brassica campestris* from the seedling stage to flowering, Chakravarti (1953) reports an increase in depth of the tunica with advancing age. In the seedling apex the files of cells of the rib-meristem converge towards a central zone of deeply staining cells. This interesting feature disappeared in seedlings more than twenty-four hours old.

A very interesting new concept has been introduced by Buffel (1949) who compares the organization of vegetative and reproductive apices. Grégoire (1938) originally contrasted the structure of the apex in the vegetative and reproductive stages of its growth, comparing the bulky corpus of the former with the domed mantle of the latter. Philipson (1947b) has described the course of the transition from one to the other in *Succisa*, and points out that the corpus is not lost, but remains as the inner part of the mantle, having lost its differentiation into zones. Buffel suggests that the central part of the corpus retains its meristematic character since he found its mitotic index to be little less than that of the mantle. Its high degree of vacuolation, however, masks its meristematic nature and throws the mantle into relief. Lawalree (1948) also compares vegetative and reproductive apices, investigating several species of the Compositae. He states (p. 233) that the transformation of the vegetative apex into the inflorescences rudiment is begun by a rapid increase in the rate of central vacuolation. He believes that the increase in size of the apex, which is associated with the production of a capitulum, does not begin until the mantle has replaced the vegetative organization. This conflict with the findings of Philipson (1946) in *Bellis*, where traces of a lens-shaped corpus were visible in very young capitula, may perhaps be explained by Buffel's findings.

Undoubtedly, one of the most significant studies of the angiosperm shoot apex is that published by Rouffa and Gunckel (1951a) on the Rosaceae. As a result of a comprehensive survey of the apex in the tribes of this large family, several important generalizations are reached. For example, after describing the variable depth of the tunica they conclude that there is no correlation between the height of the apex and the number of tunica layers, nor did they find the number of tunica layers of specific ontogenetic or taxonomic significance, though general trends in apical structure might characterize tribes or sub-families, or perhaps indicate family relationships.

From the standpoint of the present review the most important conclusion of Rouffa and Gunckel is that zonation within the apex is not a characteristic feature of the Rosaceae. Central zones were recognized by them in only five of the many species studied. This is clearly a most significant finding. As it is contrary to so many descriptions of other apices, until it is confirmed, the possibility that it is due to differences of interpretation should not be dismissed. Unfortunately, of the six photomicrographs published in this paper, five are of the species regarded as possessing a central

zone. The sixth photograph, of *Prinsepia uniflora*, has a type of apex described as unlike others in its simplicity and general lack of organization. In a subsequent paper, however, the same authors (Rouffa & Gunckel, 1951b) publish photomicrographs of the apex of *Spiraea latifolia*, one of the species considered to lack zonation. While it is not possible to decide a question of this kind by the examination of photographs, Figs. 11 and 12, which appear to be of the same apex, suggest an organization that can be interpreted as a central zone flanked by a peripheral zone. The authors label the latter, no doubt perfectly correctly, as a region of leaf initiation, but as leaf primordia first become visible in the peripheral meristem this does not preclude an interpretation of the apex as being zoned.

In this second paper, Rouffa and Gunckel (1951 b) describe the initiation of leaves in the Rosaceae. They find that there is no significant fluctuation in the stratification of the tunica as the apex passes from maximal to minimal area during the course of a plastochron, although the number of oblique and periclinal divisions in the tunica seems much more frequent at minimal area, particularly in the flanks. An original series of observations concerns the nature of the rib-meristem, and of the derivatives of it that form the pith. Two types of rib-meristem are described, but these were found to be unrelated to the type of mature pith to which they give rise. Differences in the pith rib-meristem of perennating buds appear to have no significance, since, during spring growth, similar types of pith may develop from rib-meristem of different types.

Since the nature of the mature pith is related to the habit of the shoot (e.g. whether a long or a short shoot) and not to the nature of the rib-meristem, the authors conclude that it is the derivatives of the apex rather than features of the apex itself which are correlated with habit. They disagree, therefore, with a suggestion of Philipson (1947b) that differences in apical organization will be found to be more characteristic of habit types than of taxonomic groups. In restating this criticism towards the end of their second paper (p. 306) they attribute to Philipson the view that the configuration of the apex may be important "as a diagnostic feature for taxonomic and phylogenetic affinities". This appears to be diametrically opposed to the view that habit may be reflected in apical organization. It is then claimed that the development of the derivations of the pith rib-meristem may provide a better diagnostic feature, presumably for taxons, though it is with habit, not phylogenetic affinities, that they have shown pith types to be correlated. A review of the facts presented in these two papers seems to support the view that apical organization may supply useful taxonomic characters, whereas more mature characters of the pith are correlated with growth-habit and with seasonal change. These, however, are not the conclusions drawn by the authors.

The relationship between the organization of the apex of angiosperms and other major plant groups has been discussed in two important papers. Johnson (1950) discusses the organization of the apex of *Gnetum*, which resembles that of angiosperms in the virtual absence of periclinal divisions in the surface layer. The elimination of such divisions is a minor trend in gymnosperms as a whole, but is a major trend in angiosperms, particularly in dicotyledons. The essential similarity between the zonation of angiosperms and gymnosperms pointed out by Philipson (1948) is discussed and Johnson states that "a central region of permanently embryonic tissue extending from the surface of the apex to varying depths within the shoot and from which the remainder of the apex is ultimately derived is a feature apparently possessed by all vascular plants". She concludes that "position and contribution to the remainder of the apex support this hypothesis (that the central zones of Angiosperms and Gymnosperms are homologous)", and that "the various physiological and structural expressions in this zone may properly be regarded as a matter of degree and detail".

The principal variations described for gymnosperms and angiosperms are then

enumerated. The former require no comment here, but the four types recorded for angiosperms are of interest. The first and fourth types differ only in the extent of the central zone. In the first this is restricted to the corpus and in the fourth it also includes the tunica. Earlier in this paper I have explained that the implied restriction of the central zone to the corpus in my earlier papers was unintentional and that I would unite these two types into a single category. The second type comprises a peculiar central zone of small cells observed in lateral apices of *Succisa* (Philipson, 1947a). In a subsequent paper (Philipson, 1947b) this appearance was explained as due to the transition from vegetative to reproductive growth. This peculiarity, therefore, being purely ontogenetic, should not be considered as a distinct type of apex. The third type includes the apex of *Lupinus albus* (Ball, 1949) in which the central zone is composed of blocks of cells enclosed within the original wall of the mother cell. This feature may be found to be of sufficient importance to differentiate a distinct type of apex but, at present, it seems preferable to follow Popham (1951) and regard it as a variation in detail of the normal type of organization. It is, therefore, considered that all four types may be reduced to a single basic pattern of apical organization.

A comprehensive comparison of apical organization in vascular plants has been published by Popham (1951). This author recognizes two types of apex among angiosperms. That considered as usual in flowering plants is described as having four zones. The names applied to these zones require some explanation as some are used in different senses to those adopted in this paper: (i) the *mantle* consists of the surface layer or layers in which most divisions are anticlinal; (ii) the *sub-apical initials* occupy the central position of the apex, below the mantle, and are usually relatively large, lightly straining cells, which divide relatively slowly; (iii) the *central meristem* lies below the sub-apical initials and gives rise to the pith by transverse divisions of its cells; and (iv) the *peripheral meristem* surrounds the central meristem and its cells give rise to the cortex and procambial strands.

The zonation advocated in the present paper does not differ very essentially from this, but by omitting the *mantle* the zones are reduced to three. The terms employed, however, are different: for sub-apical initials the term central zone is used, and this zone is considered to reach the surface of the apex (i.e. to include the central part of the *mantle*); for central-meristem the term rib-meristem would be used in appropriate cases; the term peripheral meristem would be extended to include the flanks of the mantle. Standard terminology, though unimportant in itself, is of value in reducing confusion and misunderstanding. The possibilities of confusion seem to be illustrated by the following sentence from a paper by Popham and Chan (1950) where they state that " derivatives of the peripheral and central mother cells become Grégoire's *manchon meristematique* and form a meristem two to five cells deep underlying the mantle "; it is suggested that the term mantle be reserved, as has been customary, for Grégoire's *manchon*.

Popham also remarks on the most significant difference between angiosperms and gymnosperms being the absence or great rarity of a tunica in the latter. Since such diverse types of plant result from apices with similar organization, he concludes that stem structure is not obviously (if at all) related to shoot apex structure.

The other type of apical organization recognized by Popham as occurring in the angiosperms differs from the usual type in possessing a rather regular zone of cambium-like cells underlying the central mother cells (which correspond very closely to the sub-apical initials of his typical dicotyledonous apex). This feature has been recognized in very few plants, those listed by Popham comprising three palms, two cacti, two Compositae and *Liriodendron*. This arrangement of zones is designated the Opuntea type of apex by Popham. Occurring as it does in such diverse angiosperms, it is very doubtful what significance this zone may possess. At least in the two Compositae

(*Bellis*, Philipson, 1946; *Chrysanthemum*, Popham & Chan, 1950) the cambium-like zone is not a constant feature, but becomes visible during the early phase of each plastochron. The appearance, therefore, may be due to an intermittent growth rate of the apex as each leaf and its internode are added to the axis. It was noticed in *Bellis* that the plate of divisions which forms the cambium-like zone extends across the axis at the level of the insertion of a leaf primordium and, therefore, occupies the position of the future node. The vegetative apex of plants in which the nodal plate is well developed in the adult plant were accordingly investigated and the Umbellifer, *Angelica sylvestris*, was found to show this feature very clearly. In Fig. 1 the cambium-like zone is visible running across the apex from the level of insertion of a leaf primordium and it can be seen that part of the zone persists at each more mature node. It is suggested that the cambium-like zone, at least in some of the plants which show it, is a specific variation of the more uniform rib-meristem, and that its development is linked either with varying rates of elongation of the axis during each plastochron or with the marked development of the node, or with both of these features.

In view of the importance of the apex as the centre of organization for the shoot,

FIG. 1 — Longitudinal section through the shoot apex of *Angelica sylvestris*. Successive leaf primordia are numbered 1-5, and the corresponding nodal plates are lettered *a-e*.

it has been employed in investigations designed to clarify problems of specific growth patterns. As a result of morphological investigations, Majumdar (1948) concludes that the procambial strands precede the leaf primordia in development and that the position of the latter, and therefore, the pattern of phyllotaxis, is determined by the growth pattern of the vascular tissue. Hegedüs (1949), while not primarily concerned with this problem, found that there was no correlation between phyllotaxis and the mode of development of the pro-vascular strands.

Experiments reported by Ball and Snow do not support the conclusion of Majumdar, and assign a more important controlling influence to the apical meristem itself.

In a series of papers, Ball (1948; 1949; 1950a, b; 1951; 1952a, b) describes experiments in which apices of *Lupinus* and *Trapaeolum* were subjected to surgical treatment, treated with growth substances, or cultured *in vitro*. The following conclusions, among others, were reached: (i) changes induced in the apex result in alterations in the subjacent tissues to which it gives rise; (ii) very small portions of the apex, when cultured *in vitro*, are able to reproduce the complete characters of the mature plant; (iii) apices isolated from subjacent vascular tissues by longitudinal cuts are able to resume normal growth; (iv) apical dominance is retained even if the central cells are destroyed; and (v) segments of the apex become reorganized to form complete apices, and their subsequent growth produces complete axes.

Earlier experimental work by Snow and Snow (1947) had already thrown much light on the problem of phyllotaxis. In a previous paper (Philipson, 1948) the basis of one of their experiments was discussed, and one of the authors (Snow, 1951) has replied briefly in a footnote. As the criticism appears to have been misunderstood, a further brief explanation may be of use. It was not questioned that the experiments in which vertical cuts displaced subsequent leaf primordia provide evidence of the time at which primordia are determined, and this is so whether this displacement of the primordia is due to interference with its

area or to displacement of the space available for its growth. It was suggested, however, that even at the early stage when the growth of a primordium might be displaced by surgical means, its position almost certainly already had been selected and marked by cell divisions. Horizontal cuts below such portions of the apex, therefore, could not prove that phyllotaxis was independent of the position of procambial strands because the pattern of the primordia was already present above the cut. This suggestion, which was based on experience of apices of other species, has been proved correct by a figure of the apex of the material in question (*Lupinus*) subsequently published by Ball (1949, Fig. 6). In this figure the position of a leaf primordium is defined at a level clearly above that of the horizontal cuts described by Snow and Snow (1947).

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NEMALIONOPSIS IN AMERICA

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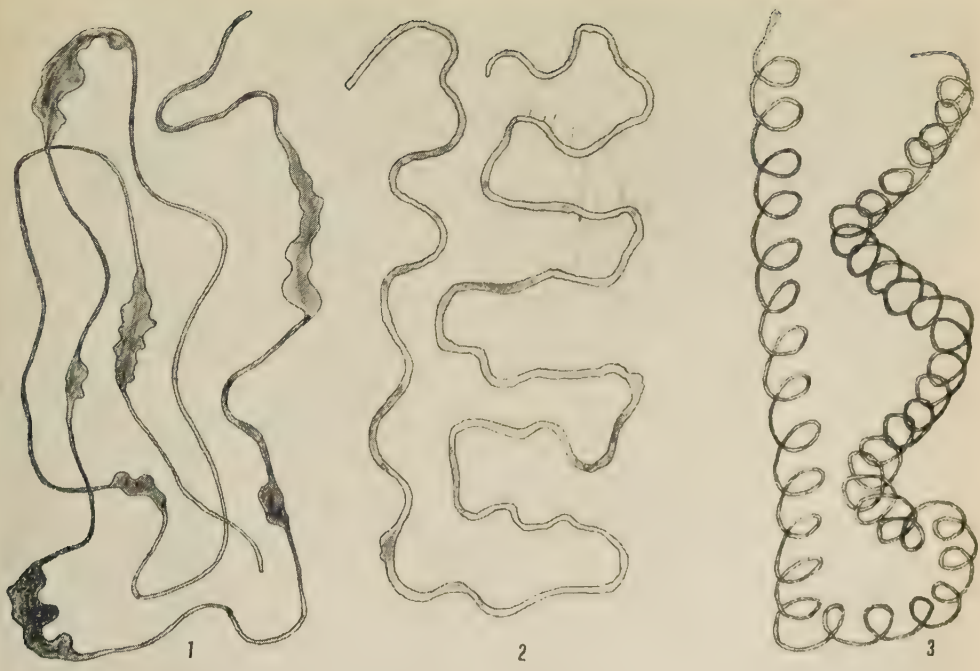
In 1934 the genus *Nemalionopsis* was established by Skuja to accommodate a freshwater red alga found in the Philippine Islands, which he designated as *N. shawi*. The plant, while having obvious resemblances to species of the marine genus *Nemalion*, also resembled the freshwater genus *Thorea*. In the second volume by Fritsch (1945) on the structure and reproduction of the algae, these two genera have been placed in the family Thoreaceae in the order Nemalionales.

On October 20 1951, in the course of a somewhat intensive survey made possible by a research grant from Louisiana State University, the writer collected specimens of a plant which appeared to be "a different species of Thorea" in a stream about thirty miles west of Alexandria, in Rapides Parish, Louisiana. The stream was a tributary of the Calcasieu river. The plants were attached to submerged logs in swift water. Other red algae in the same stream and habitat were *Compsopogon coeruleus* (Balbis) Mont., *Batrachospermum moniliforme* Roth., *B. sirodotii* Skuja, *B. globosporum* Israels., and *B. mikrogyne* Flint et Skuja. On October 27, 1951, the stream was revisited and the plants collected at this time included the Chantransia stage and young plants originating therein. On March 22, 1952, the stream was visited again, and on this occasion no macroscopic material was present. Young plants of the Thorea-like form were found on pieces of substrate removed and examined with a binocular microscope in the laboratory. These structures were reddish brown cones a few millimeters in height and diameter. It thus was made obvious that the plant would have been overlooked in collections made during the spring months when most species of freshwater red algae attain their greatest luxuriance.

From the laboratory study of the plant material it became obvious that the plant was not *Thorea ramossissima* Bory found in several states of this country and of widespread distribution throughout the world. Specimens were sent to Dr. Skuja at Uppsala, Sweden, who identified the plant as *Nemalionopsis*, probably a form of *N. shawi* Skuja. On October 22, 1952, the stream was revisited and additional material was collected. Based on the study of the plants collected as indicated, the following description has been derived.

Description of American Nemalionopsis

The Louisiana plants consisted of three types of fronds; (i) olive, brown or yellow olive cord-like strands with numerous flattened portions having wavy margins, to 50 cm. in length, shown in Fig. 1; (ii) olivaceous, irregular cord-like strands somewhat larger in diameter than the foregoing, characterized by the presence of numerous short whitish outgrowths at right angles to the main axis, to 50 cm. in length, shown in Fig. 2; (iii) reddish brown, smooth cord-like strands, often coiled, diameter as in the first type, attaining lengths up to 100 cm., shown in Fig. 3. Unsuccessful attempts were made to attach some genetic significance to the presence of the three types of fronds. Although some differences were noted, such as the sparse production of monospores on the flattened portions of the type shown in Fig. 1, no real distinction in function could be made. Monospores, shown in Fig. 6, were produced freely, not only on the three types of plants, but on the short whitish lateral outgrowths (Figs. 2, 4) and on the very young plants (Fig. 8) which were found in the branches of the Chantransia stage (Fig. 13). In the latter situation the



FIGS. 1-3 — Fig. 1. Type of frond characterized by the presence of flattened portions. Fig. 2. Type of frond characterized by the presence of short whitish laterals at right angles to the main axis. Fig. 3. Type of frond characterized as a smooth cord and a strong tendency to coil. All figures approximately natural size.

filaments as well as the monospores were smaller and of a slate-green color, but otherwise were identical (Fig. 9). Often the persistence of transparent ruptured monosporangial walls (Fig. 6) evidenced that a succession of monospores had been produced and released. In other instances it was clear that a simple extension of the strand had followed the production of one or more terminal monospores (Fig. 6, lower unit).

The older portions of the cord-like structures consisted of a central multiaxial core of elongated, branched, interwoven rhizoidal and mostly colorless strands. A cross-section is shown in Fig. 11. This core was surrounded by a looser spongy mass of rhizoidal strands, from the outer portion of which there developed (Fig. 5) compact masses of short, more or less closely tufted filaments of pigmented cells extending radiately and contiguously to comprise a firm cortex (Fig. 11). The pigmented cells contained several strap-shaped parietal-olivaceous to brown plastids (Fig. 6). In the event of filament maceration the cortical tufts under slight pressure of the cover glass on a microscope slide became oriented as symmetrical discoid structures (Fig. 12). *In situ* germination of the monospores (Fig. 7) was of common occurrence and the rhizoidal strands which developed subsequently became a part of the frond complex. Within the pigmented cells the small nucleus was central or adnate to the wall in the middle region, minute starch grains often were free in the cytoplasm or attached to the surfaces of the plastids and protoplasmic connections were obvious.

FIGS. 4-13 — Fig. 4. Portion of frond shown in Fig. 2. × 50. Fig. 5. Basal portion of cortical tuft. × 200. Fig. 6. Portions of cortical filaments showing monosporangia and wall vestiges. × 400. Fig. 7. Germinating monospore. × 400. Fig. 8. Two young plants from within chantransial tufts. × 50. Fig. 9. Monosporangium borne in structures shown in Fig. 8. × 400. Fig. 10. Structure suggestive of a cystocarp. × 400. Fig. 11. Cross-section of adult frond. × 15. Fig. 12. Whorls derived upon maceration of frond cortex. × 25. Fig. 13. Portion of chantransial plant. × 400. All magnifications approximate.

The fronds were attached to the substrate by claw-like proliferated structures, for the most part pigmented where exposed to light, but lacking extensive monospore production. All plants were sharply restricted to swift water and the major growth took place during the summer months. In October there was an abundance of chantransial plants on the adjacent substrates, these plants having the same general characteristics of the tufted pigmented cortical filaments, including monospores. They attained a height of 4 mm. Several minute structures representing early stages of the described fronds were found within the chantransial tufts. Two of these have been indicated in Fig. 8. These young plants were differentiated readily by a contrasting slate-green color. Even on these early stages, however, monospores were being produced.

The finding of a single structure suggestive of a cystocarp, of a deep yellow brown color and bearing at its base two structures which resembled trichogynes with attached antheridian vestiges (Fig. 10) gave rise to an extensive search for additional corroborative structures. These were not found, however, and for the present the nature of the sexual organs must remain in doubt. Rarely there were observed structures having the general appearance of monospores but with two or four internal protoplasmic masses. Although these suggested tetrasporic development, their occurrence was so infrequent as to deprecate the possibility.

Discussion

The presence of three types of fronds in the Louisiana material enhanced confusion, since plants of the third type as listed and described closely resembled *Thorea* except for the absence of a loose outer cortex. On account of this resemblance Dr. Francis Drouet, of the Chicago Museum of Natural History, was asked to examine the sheets of *Thorea* in the Cryptogamic Herbarium with special reference to plants devoid of the loose outer cortex. This search led to the discovery of a single number having fronds with a smooth cortex: plants collected in Mexico near Santa Catarina, Nuevo Leon, by F. A. Barkley on August 16, 1944. A comparison of these plants with plants of the third type (Fig. 3) of the Louisiana collections suggested that they might be of the same species.

By analogy with other freshwater red algae in which a chantransoid stage has been recognized there would appear to be a strong probability that the macroscopic fronds of both *Thorea* and *Nemalionopsis* are sexual plants, even though sexual organs have not as yet been reported and described in a satisfactory manner. A similar situation holds for the genus *Compsopogon*, In relation to plants of these three genera — *Nemalionopsis*, *Thorea* and *Compsopogon* — and the incidence of sexual organs it may be of interest to point out that whereas in Louisiana the reproduction of *Batrachospermum vagum* is mostly by monospores, one collection consisted entirely of plants reproducing sexually. Thus there is the suggestion that additional collections may supply supplementary items not only for the description of *Nemalionopsis*, but also for *Thorea* and *Compsopogon*. Until the sexual organs in these plants have been studied in extensive detail the descriptions must remain to an appreciable extent inadequate and unsatisfactory.

Summary

In 1934 the genus *Nemalionopsis* was established by Skuja to accommodate a freshwater red alga from the Philippine Islands which he designated as *N. shawi*, and since that time no other species of the genus has been described. This paper reports the discovery in the United States of plants of this genus and describes the material which has been found to date in Louisiana. The description is in general accord with that of the Philippine plant and the American plant is considered, therefore, as a form of *N. shawi* Skuja, family Thoreaceae, order Nemalionales.

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A CONTRIBUTION TO THE EMBRYOLOGY OF *UTRICULARIA FLEXUOSA* VAHL

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Introduction

The plants belonging to the family Lentibulariaceae have attracted attention for a long time for a variety of reasons. Their carnivorous habit has been the centre of special interest and the bladders of *Utricularia* are particularly well known. They have been a fascinating object of study and have inspired many researches. The embryological study of these plants has also revealed a number of noteworthy features. The presence of nutritive tissue in the ovule and the placenta, the embryo sac growing out of the ovule into the ovarian cavity and entering the placental nutritive tissue, the pollen tube meeting the embryo sac outside the ovule, the endosperm haustoria, the development of a long tube from the zygote and lack of differentiation in the mature embryo are all normal features of *Utricularia*. Some of these are shared more or less by other members of the family as well.

In comparison with the morphological and physiological studies, investigations on the embryological aspects of these plants have, however, been less impressive. Kausik's (1938) work on *U. coerulea* is the only contribution to our knowledge of the embryology of the Indian species of *Utricularia*. The remarkable features of the genus, the occurrence of many species in India and the almost complete neglect with which the latter have so far been treated, prompted me to take up a study of these fascinating plants. The present work is mainly devoted to one species, namely, *U. flexuosa* Vahl although occasional reference is also made to *U. stellaris* var. *inflexa* Clarke.

Historical

The family comprises five genera (Kamienski, 1897; see also Barnhart, 1916) of which *Utricularia* alone has been studied in some detail. The work on *Pinguicula*, *Polypompholyx* and *Genlisea* is rather scanty, while *Biovularia* has not been studied so far. The earlier work includes the contributions of Kamienski (1877), Merz (1897), Lang (1901), Merl (1915) and Wylie and Yocom (1923) (see also Samuelsson, 1913 and Jacobsson-Stiasny, 1914). These have been briefly reviewed by Schürhoff (1926) and Schnarf (1931). The only publications, after Schnarf's review, are those of Kausik (1938) and Stolt (1936). It is proposed to refer to the relevant literature in various sections of the text as occasion demands and, therefore, a review of previous work is being omitted here.

Material and Methods

The material of *Utricularia flexuosa* was collected from the Najafgarh drain (Delhi) in November 1949 and in January and October 1951. Some material of this species, collected from Dacca (East Bengal) in August 1950, was kindly sent to me by Mr. A. M. Eunus. The material of *U. stellaris* was kindly collected by Mr. B. Tiagi from Meerut. Further specimens of *U. stellaris* var. *inflexa* were collected by me from the Najafgarh drain in October 1951.

Formalin-acetic-alcohol and Navashin's fluid were used as fixatives. The usual methods of dehydration, infiltration and embedding in paraffin were employed. Sections were cut at a thickness of 6 μ

for a study of microsporogenesis, 8-10 μ for megasporogenesis and development of embryo sac, 10-12 μ for endosperm and embryo and 15-20 μ for the study of seed structure. Staining was done in safranin and fast green as well as in iron alum haematoxylin. Acetocarmin smears and whole mounts were prepared from anthers and young ovaries to study the male gametophyte and young ovules. Whole mounts of pollen grains were prepared in methyl-blue glycerin jelly according to the method described by Wodehouse (1935) with the difference that methyl-green was substituted by methyl-blue.

Flower

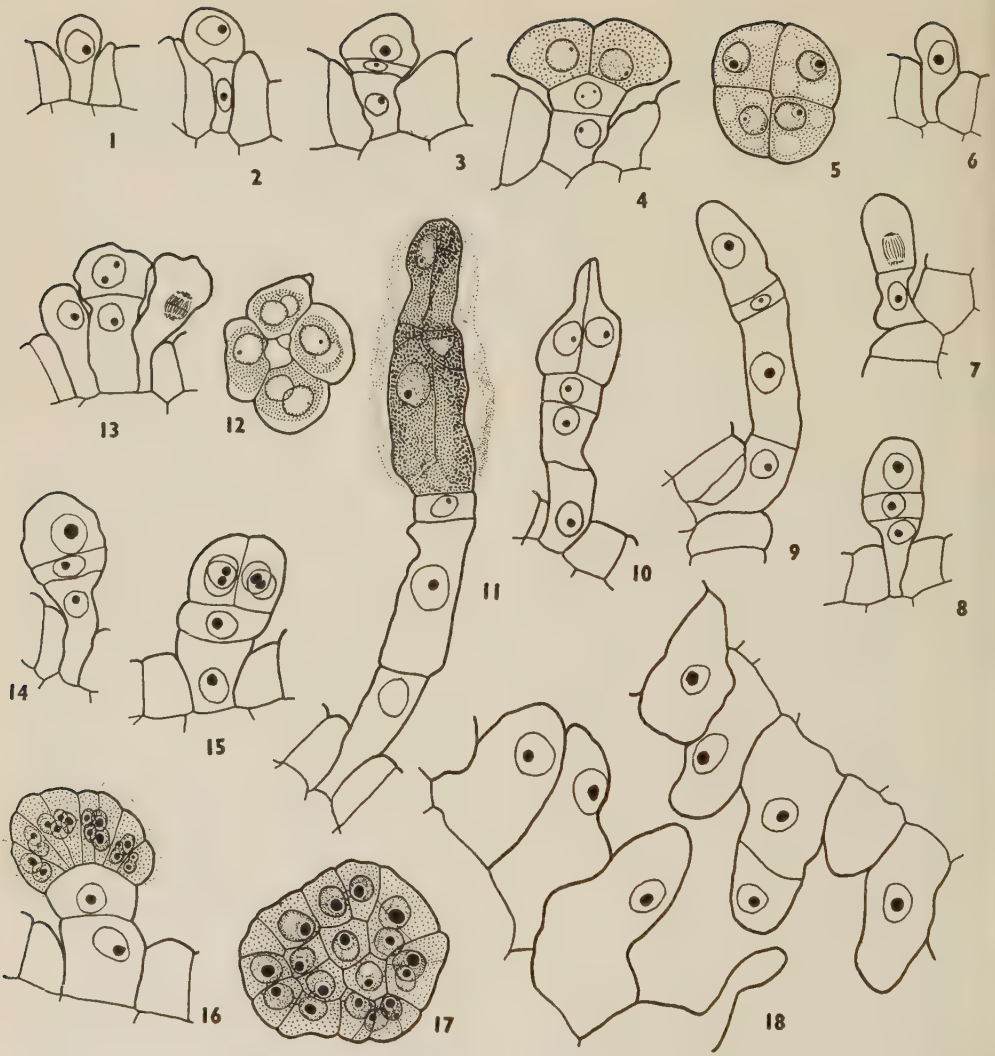
The rootless, free-floating *Utricularia flexuosa* grows submerged except for the racemose inflorescence which grows up into the air. In this species the peduncle does not usually bear the floats which are so characteristic of *U. stellaris*. The flowers arise in the axils of thin membranous bracts.

The calyx, which is persistent and accrescent, is gamosepalous, but is deeply divided into two segments which are often referred to as sepals (Figs. 19-21). The two segments are almost similar in size and form. The calyx bears on its outer surface glandular hairs which develop from epidermal cells. The cell destined to give rise to the hair protrudes out and divides transversely into two cells (Figs. 1, 2). The apical cell again divides transversely, so that the young hair now consists of a long basal cell, a short disc-like middle cell and a hemispherical apical cell (Fig. 3). The basal and the middle cells remain undivided and form the 2-celled stalk. The apical cell divides longitudinally by two walls at right angles to each other, producing four cells placed isobilaterally in one tier. These four cells constitute the gland (Figs. 4, 5). In older stages the contents of these cells become very dense. Glandular hairs of this type are also present on the pedicel.

The upper lip of the bilabiate yellow corolla is broad, semicircular and somewhat erect, looking like the standard petal of a papilionaceous corolla. The lower lip spreads horizontally or may be sloping. The corolla bears three kinds of hairs all of which differ from those on the calyx (Fig. 19). The 3-celled stage of the hairs on the outer surface is derived in the same way as in the hairs on the calyx (Figs. 6-8). The mature hair has a stalk consisting of 3 to about half a dozen cells arranged in a row. The uppermost cell of the stalk is short and disc-like, while the remaining cells are elongated (Figs. 9-11). The apical cell of the 3-celled stage gives rise to the gland which consists of a varying number of cells situated in two or more tiers (Figs. 9-12). The contents of the cells constituting the gland become dense and the gland seems to secrete a substance which closely envelops it. The cells and their contents are, therefore, not quite clear in older glands. The anterior lip of the corolla bears on its inner surface, in the region adjacent to the anthers, glandular hairs of a different type (Fig. 19). These resemble the hairs on the calyx, but the gland consists of 16 or more cells (Figs. 13-17). Occasionally the stalk may have two disc-like cells. The anterior lip of the corolla also bears 2 to 4-celled, usually uniseriate hairs on its inner surface, where it approaches the posterior lip and closes the corolla tube. Many epidermal cells on the inner surface of the corolla become elongated or assume a papillate appearance, especially in the region where the two corolla lips meet each other (Figs. 18, 19).

The two epipetalous stamens are anterior in position and stand side by side. The anthers are situated just below the stigmatic lip which is arched over them. Each anther usually contains four pollen chambers, but occasionally it may have five.

The gynaeceum is flask-shaped and has a hollow style (Figs. 19-21). The upper stigmatic lip is extremely inconspicuous and may be described as absent. The lower or the anterior lip is well developed and bears a large number of stigmatic papillae. The latter have a broad base and gradually taper towards the tip. The outer surface of the gynaeceum bears glandular hairs like those seen on the calyx. The ovary is unilocular (Fig. 20). On the inner surface of the wall of ovary are occasionally seen glandular hairs of the



FIGS. 1-18 — *Utricularia flexuosa*. Figs. 1-5. Development of glandular hairs on the calyx. In Fig. 5 the gland is seen in surface view. Figs. 6-12. Development of glandular hairs on outer surface of corolla. Fig. 12 shows the gland in t.s. Figs. 13-17. Development of glandular hairs on the inner surface of corolla. The gland is seen in t.s. in Fig. 17. Fig. 18. Cells of the inner epidermis of the two lips of corolla in the region where the lips meet to close the corolla tube. One cell has divided transversely. × 832.

type described as occurring on the inner surface of the corolla in the region adjacent to the anthers. From the base of the ovarian cavity arises a stalk bearing the placenta which is massive and spherical except for a depression at the basal pole into which the stalk is attached. The ovules are very small and indefinite in number and occupy almost the entire surface of the placenta. The placenta also bears, near the base, glandular hairs which are of the type seen on the inner surface of the ovary wall or the inner surface of the corolla.

The form of the fruit exhibits a certain degree of variation. Specimens collected from Dacca frequently differed from Delhi specimens in having fruit which, together with its stalk, is more slender and in which the transition between the ovary and style is more gradual. When this point was referred to the Director, Royal Botanic Gardens, Kew, he replied, quoting Mr. P. Taylor, who is working intensively on the genus with a view to an eventual revision, as follows: " The fruits of the Kew material of *U. flexuosa* Vahl vary considerably in shape and size, and the two specimens D^1 and E^1 are well within the range of variation. It is quite possible, however, that more than one species is at present included under that name. The seeds of D are much larger than those of E, but this character again varies similarly in the Kew material." Since the present study is based entirely on material collected at Delhi, there is not much likelihood of any confusion even if it is later confirmed that the name *U. flexuosa*, as understood at present, covers more than one species.

The characteristic feature of *U. stellaris* is the presence of floats near the base of the peduncle. The corolla is yellow in *U. stellaris* but in *U. stellaris* var. *inflexa*, it is white with violet streaks.

Organogeny

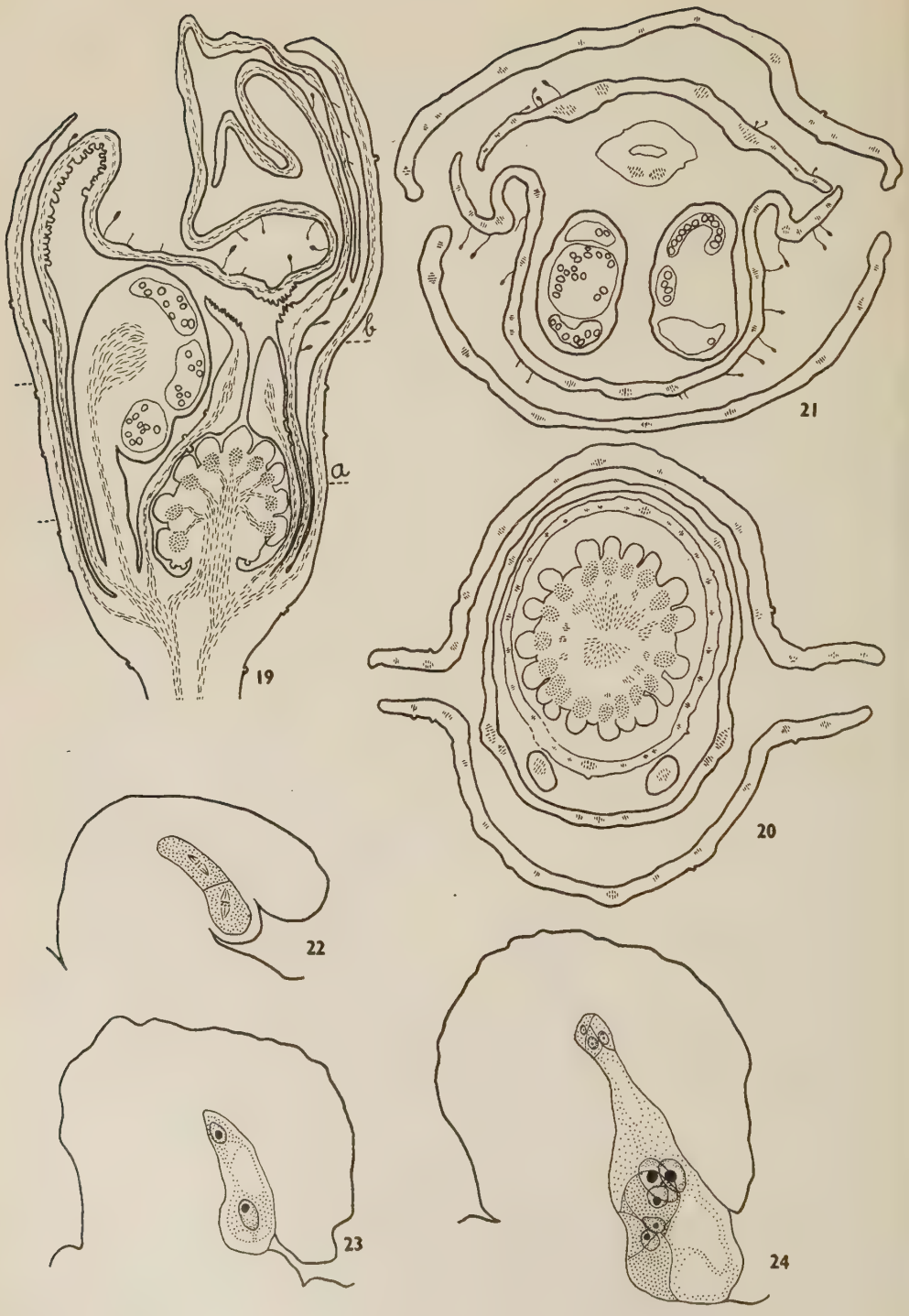
The floral primordium makes its appearance as a dome-shaped mass of cells, protruding from the axis of the young inflorescence a little below its apex and situated in the axil of a bract which is itself in the initial stage of development. A young flower bud shows the rudiments of the calyx and corolla at the base followed by the primordia of the stamens and then the convex apical region which differentiates into the gynaeceum. The first sign of development is the appearance of an outgrowth on the anterior side of the apex. It later extends to the other side of the convex area. This outgrowth is the beginning of the wall of the ovary while the convex apex itself develops into the placenta. Since the ovary wall originates first on the anterior side, it is larger on this side. The difference is maintained till the end and becomes even more conspicuous by the differentiation of the stigma on the anterior side only. The ovary wall, as it grows, converges towards the centre and covers the young placenta. It does not, however, close up completely but leaves a narrow passage at the apex which forms the hollow stylar canal.

Kausik (1938) says that in *Utricularia coerulea* the origin and development of the floral parts proceed in the usual manner. This is followed by the following statement: " The sepals are the first to make their appearance. The ovary is differentiated next as a conical mass of cells terminating the floral axis. All round this cone and within the whorl of sepals, the primordia of the petals arise, which rapidly grow in size and fold over the central cone. The stamens are the last to appear and take their origin on the inside of the petals almost at the base. Their enlarged distal portions later become the anthers (Figs. 2-4). A ring of cells arises from the base of the central cone and after considerable growth forms the ovary wall, while the cone itself becomes the massive placenta." In spite of an apparent difference, the statement seems to suggest that the gynaeceum of *U. coerulea* differentiates last of all as described here in *U. flexuosa*.

Megasporangium

The ovules are anatropous, unitegmic and tenuinucellate. Occasional hemianatropous ovules have been noted. In longitudinal sections of the gynaeceum the ovules are seen to be arranged one behind the other except at the apex of the placenta where they are situated back to back. Occasionally an ovule shows the reverse orientation, resulting in a face-to-face disposition. The youngest ovules are near the base of the placenta, the oldest at the top. During the growth of the ovule there differentiates a group of cells near its base constituting the placental nutritive tissue. The cells have dense protoplasmic contents and are

1. D = Delhi specimens; E = Dacca specimens.



FIGS. 19-24.

organized into a compact ovoid mass which forms a distinctive feature of the placenta whether in transverse or in longitudinal section. In older stages each mass is seen to be surrounded by a sheath of two or three layers of cells. The vascular supply of the placenta, as seen in longitudinal section, appears in the form of a "tree" (see Wylie & Yocom, 1923). Near the base of the placenta, the vascular strands form a column comparable to the trunk of the tree. At a higher level the strands undergo branching. The ultimate ramifications approach the groups of nutritive cells in the peripheral region. Thus the nutritive cells do not form isolated islands but are in communication with the vascular supply of the placenta (Fig. 83).

The ovule is at first a simple protuberance of cells (Figs. 25, 26). As it grows and bends downwards, the integument arises on its convex side (Fig. 27). It grows rapidly and extends beyond the nucellus. As the ovule becomes anatropous, the wide micropyle becomes directed towards the placenta. The micropyle has only a temporary existence, however. Since the nucellar epidermis degenerates at an early stage, the developing embryo sac, which is now naked, grows beyond the integument, its apex coming in direct contact with the placenta (Figs. 22-24). At this stage, therefore, the ovule may well be said to have no micropyle at all. In one ovary, containing ovules with mostly functional megaspores or 2-nucleate embryo sacs, almost all the sacs were protruding far beyond the integument. Some of them had come in contact with adjacent ovules while others had touched the ovary wall. This lack of a micropyle is due, in some measure, to an early enlargement of the embryo sac but mainly to arrested development of the integument.

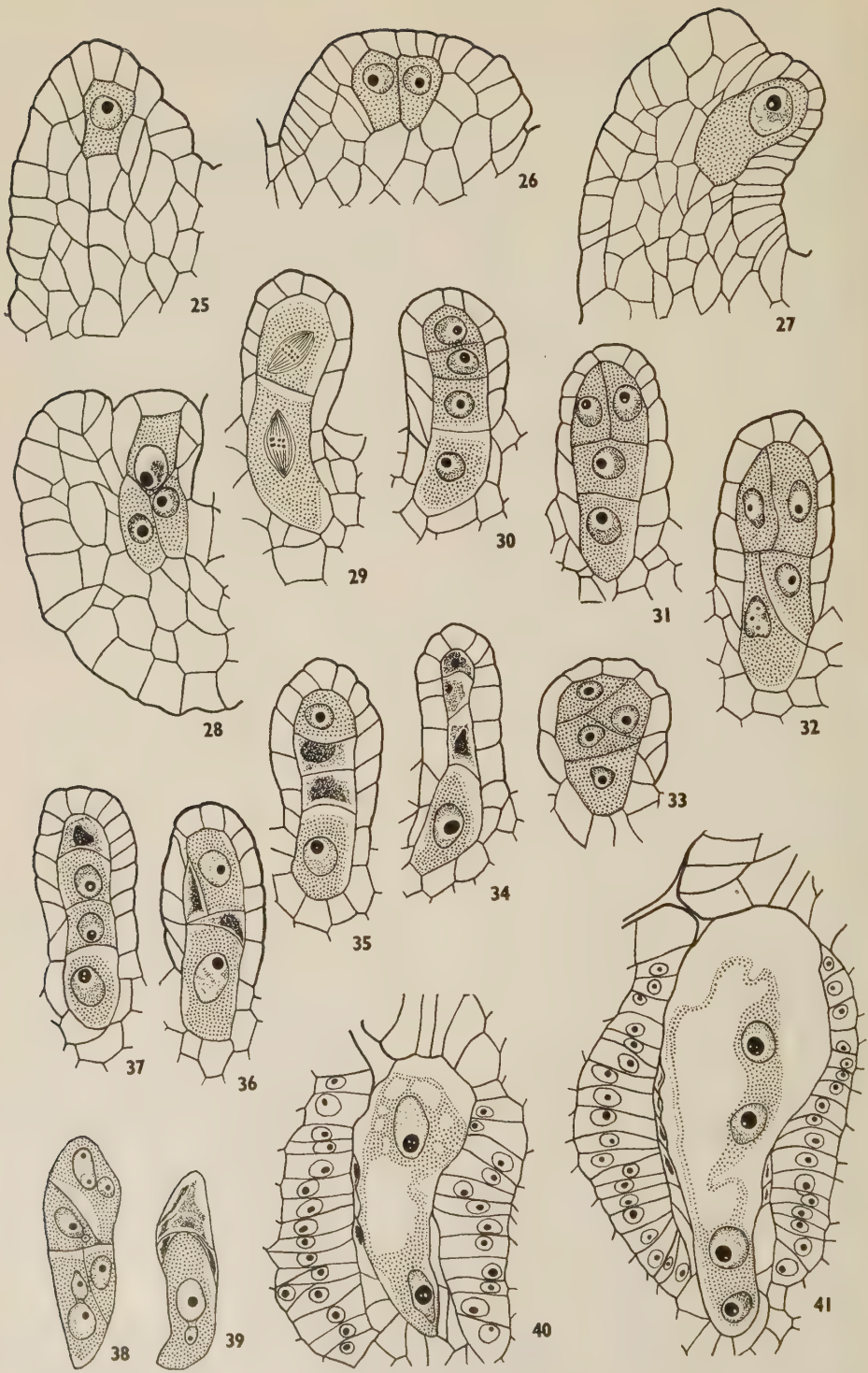
The innermost layer of the integument differentiates into a prominent endothelium whose cells have prominent nuclei and dense cytoplasm (Figs. 40-42). In longitudinal sections of the ovule they are elongated at right angles to the embryo sac. In transverse sections of the ovule, they are polygonal and isodiametric. The endothelium comes in direct contact with the embryo sac except in the chalazal region where one layer of cells separates the embryo sac from the lower cells of the endothelium (Fig. 42). In transverse sections of the ovule the cells of the endothelium very often give the impression of containing more than one nucleus, but it is not possible to be sure of this because overlapping cells can also produce similar appearances. This possibility is particularly noteworthy in transverse sections of the ovule because the cells of endothelium are so narrow in this plane.

In mature ovules a group of cells in the chalazal region around the base of the embryo sac can usually be distinguished from the neighbouring cells by the smaller size of the former. This is the chalazal nutritive tissue. It does not have a definite shape or organization (Fig. 42).

In *U. vulgaris americana*, as in *U. flexuosa*, the whole surface of the spherical placenta is studded with ovules. However, in *U. coerulea* and some species of *Genlisea* (Merl, 1915) the apical region of the placenta does not bear any ovules. The occurrence of a placental nutritive tissue has been reported in many species of *Utricularia* (Merz, 1897; Merl, 1915; Kausik, 1938). A somewhat similar tissue occurs in *Polypompholyx* (Lang, 1901) at the base of the funicle, and in *Genlisea* (Merl, 1915) inside the ovule in the integument near the micropylar end of the embryo sac.

As in other species of *Utricularia*, the ovules of *U. flexuosa* lack a vascular supply. This seems to be associated with the fact that the embryo sac obtains its nourishment directly from the placental nutritive tissue without its passing through the funicle. In older stages the funicle is not required either for transmission of

FIGS. 19-24 — *Utricularia flexuosa*. Fig. 19. Diagrammatic sketch of flower bud in l.s. Note the position of glandular hairs on different organs. Figs. 20, 21. Flower bud in t.s. at the levels *a* and *b* respectively, shown in Fig. 19. Figs. 22-24. Stages in development of ovule. Figs. 19-21. × 43. Figs. 22-24. × 436.



FIGS. 25-41.

food or for attachment, the latter function also being performed, at least in part, by the micropylar endosperm haustorium which is deeply embedded in the placenta.

ARCHESPORIUM AND MEGASPOROGENESIS — There is usually a single hypodermal archesporial initial cell (Fig. 25), but occasionally two or three may be present (Figs. 26, 28). The archesporial cell functions directly as the megaspore mother cell which is often slightly curved (Fig. 27). The megaspore mother cell gives rise to two dyad cells which divide simultaneously to produce four megaspores (Fig. 29). The megaspore tetrad is remarkable for the variation in its form. Usually it is of the linear type (Fig. 30). Frequently it is T-shaped or the wall separating the two upper megaspores is oblique (Fig. 31). Sometimes the arrangement is isobilateral (Figs. 32, 38). Occasionally the two middle megaspores are situated side by side resulting in a crosswise arrangement (Fig. 33). In *U. stellaris* var. *inflexa* the megaspore tetrad is linear or occasionally isobilateral.

Of the four megaspores of a tetrad the chalazal usually develops into the embryo sac (Fig. 34). A few tetrads showed more than one healthy megaspore (Figs. 35-38). It seems that many cases of twin embryo sacs and of triplets or quadruplets, to be described later, arise in this way. In most cases the extra functional megaspore is usually the uppermost cell of the tetrad, but in two instances only the uppermost megaspore had degenerated while the remaining three were quite healthy. In one tetrad showing crosswise arrangement of megaspores, both the upper and lower megaspores were healthy. Of the two middle megaspores, situated side by side, one was in an advanced stage of degeneration while the other, also degenerating, had already become binucleate. Occasionally all the four megaspores remain healthy and may be regarded as potentially functional (Figs. 38, 48, 49). Some megaspores behave in a strange fashion. In an isobilateral tetrad, in which all the megaspores were healthy, the nucleus of one exhibited a bud-like outgrowth which had a separate nucleolus. Another megaspore in the same tetrad had a separate smaller nucleus in addition to a large one. One of the remaining two megaspores was also binucleate although one of the nuclei was extremely small and may be called a micronucleus (Figs. 38, 39). This condition may be the result either of unequal mitotic division caused by an irregular behaviour of the chromosomes or may be the result of amitotic division.

In *U. flexuosa* the two dyads divide simultaneously but in *U. coerulea* (Kausik, 1938) the lower dyad always divides in advance of the upper cell. The variation in the form of the megaspore tetrad as described here has not been reported in other species of *Utricularia* and is known in very few plants. Two such instances, cited by Maheshwari (1950), are those of *Poa* (Håkansson, 1943) and *Musa* (Dodds, 1945). A similar variation has recently been reported in *Elaeis guineensis* (Kajale & Ranade, 1952). The variation in the number of functional megaspores in a single tetrad seen in *U. flexuosa* has also not been reported in other members of the family Lentibulariaceae.

Female Gametophyte

DEVELOPMENT OF GAMETOPHYTE — The development of female gametophyte conforms to the Polygonum type. At the

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FIGS. 25-41 — *Utricularia flexuosa*. Fig. 25. Primordium of ovule with hypodermal archesporial initial cell. Fig. 26. Two archesporial cells in the same ovule. Fig. 27. Young ovule showing the megaspore mother cell and the initiation of the integument. Fig. 28. Three megaspore mother cells in the same ovule. Fig. 29. The two dyad cells undergoing division. Fig. 30. Linear tetrad of megaspores. Fig. 31. T-shaped tetrad. Fig. 32. Isobilateral tetrad. Fig. 33. Tetrad with the two middle megaspores situated side by side (reconstructed). Figs. 34-37. Tetrads with degenerating and functional megaspores (Fig. 37 has been reconstructed). Figs. 38, 39. Megaspores with a budding or supernumerary nucleus. In Fig. 38 all the megaspores of the tetrad are healthy. Figs. 40, 41. 2- and 4-nucleate stages of embryo sac. Most of the nucellar cells have disappeared; a few can be seen in degenerated condition. The naked embryo sac is in contact with placenta. The endothelium has become prominent. × 810.

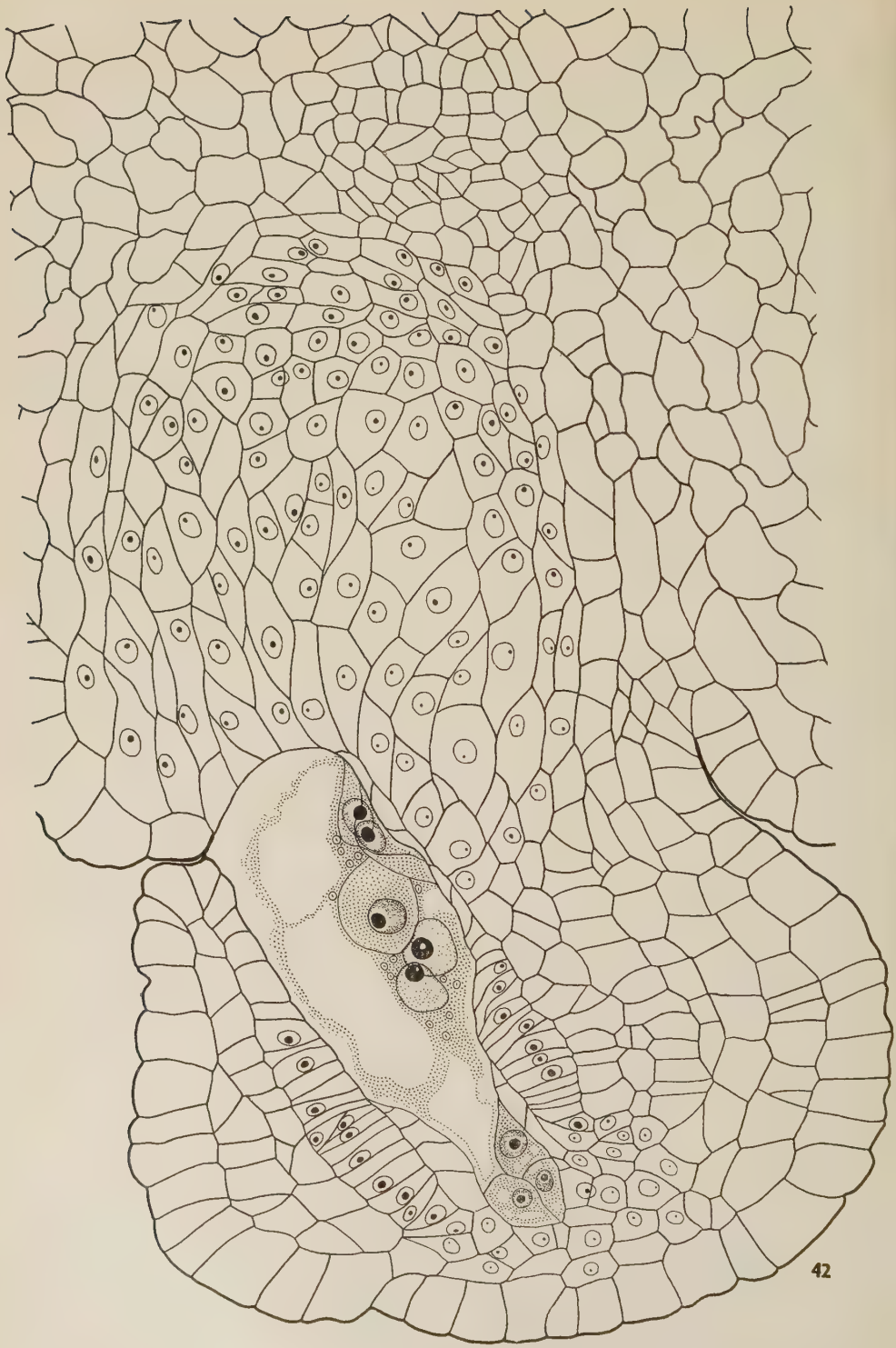

Fig. 42.

late 2-nucleate stage the apical part of the embryo sac is completely naked and in direct contact with the placenta (Figs. 23, 40). At the 4-nucleate stage the embryo sac enlarges considerably and becomes divisible into two regions, of which the lower is much smaller and narrower than the upper. The nuclei are embedded in the peripheral cytoplasmic lining on the funicular side (Fig. 41). At about this stage the apex of the embryo sac begins to penetrate into the placental tissue.

The 8-nucleate mature embryo sac ordinarily shows the usual organization (Figs. 24, 42). The egg apparatus is situated towards the funicular side of the embryo sac. The three cells are so arranged that a longitudinal section of the ovule, cut in plane showing the funicle on one side and the passage between the integument and placenta on the other, usually exhibits the egg apparatus in side view (Fig. 42). On the other hand, when a longitudinal section is cut in a plane parallel to the funicle, the egg apparatus is seen in face view, occupying a median position. The egg as well as synergids show considerable variation in shape. The synergids do not possess a filiform apparatus. One of the synergids is often seen in such a position in relation to the egg that the two seem to form a single 2-celled structure. The polar nuclei are the largest nuclei in the embryo sac. The antipodals are usually the smallest cells in the sac but sometimes they are large and contain one or two prominent vacuoles. The arrangement of the three cells shows considerable variation. Occasionally there are only two antipodal cells, one being binucleate.

The mature embryo sac has certain noteworthy features. The greater part of it is naked in the sense that it is not invested by a nucellus; the lower narrow end is embedded in the chalaza, usually in a group of nutritive cells; and the middle part is sheathed by the integumentary tapetum. The upper bulbous portion protrudes beyond the ovule into the ovarian cavity and its apex is buried in the placental nutritive tissue (Figs. 24, 42). This may, therefore, be described as haustorial. The embryo sac receives nourishment from three directions: from the chalazal nutritive tissue of the ovule, from the integumentary tapetum and from the placenta. The mature embryo sac usually contains a large number of starch grains (Figs. 43, 44, 47, 85). The embryo sac is in direct communication with the ovary cavity through a semi-circular slit lying between the integument and the placenta and with the external atmosphere through the hollow stylar canal. The slit, which is situated on one side of the sac, may be said to be analogous to the micropyle, because it is through this that the pollen tube approaches the embryo sac. However, it is not homologous to the micropyle. Sometimes the passage is wide and the exposed area of the embryo sac so extensive that even the analogy hardly holds good (Figs. 24, 84).

The Polygonum type of embryo sac is found in an overwhelming majority of the Tubiflorae. A departure has been reported only in occasional cases and some of these reports are doubtful. It seems that *Lycopsis arvensis*, *Anchusa officinalis* (Svensson, 1925), *Cuscuta reflexa* (Johri & Nand, 1934; Johri & Tiagi, 1952) and some species of *Nicotiana* (Modilewski, 1936, 1937a, 1937b; Modilewski & Dzubenko, 1937) are the only plants in Tubiflorae in which the Allium type of embryo sac is known with certainty. Although a tetrasporic development of embryo sac has been reported in a few cases in the order, no case can be regarded as established beyond doubt (see Schnarf, 1931; Maheshwari, 1937, 1941; Mauritzon, 1936; Khan, 1943).

The remarkable behaviour of the embryo sac, which grows beyond the ovule and becomes haustorial, was first reported in *Utricularia* by Merz (1897). It is

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FIG. 42 — *Utricularia flexuosa*. L.s. of ovule containing mature embryo sac, together with a part of placenta. Nuclei have been shown in the cells of placental and chalazal nutritive tissues and integumentary tapetum. Note the haustorial apex of embryo sac and the position of egg apparatus (reconstructed). × 846.

now known to be the usual feature of the genus. *Polypompholyx* (Lang, 1901), with a group of nutritive cells at the base of funicle, is another genus of the Lentibulariaceae in which the embryo sac behaves in a similar way. In *Genlisea* (Merl, 1915), in which the ovule has nutritive cells in the integument near the micropylar end of the embryo sac, and, in *Pinguicula* (Merz, 1897; Stolt, 1936), which is not known to have an organized nutritive tissue, the embryo sac remains inside the ovule. The haustorial behaviour of the embryo sac, therefore, seems to be correlated with the presence and location of the nutritive tissue.

Variation in Development and Organization of Embryo Sac — A very frequent variation in the organization of the embryo sac is the presence of supernumerary polar nuclei accompanied by a decrease in the number of synergids. More than a dozen embryo sacs show three polar nuclei and only one synergid (Fig. 43). It seems that one of the two nuclei, destined to form the synergids, moves down and becomes the third polar. The number of antipodals in these sacs was either three or fewer than three and in some cases no antipodals could be distinguished at all. Since this often happens also in embryo sacs with the usual organization, it seems to have nothing to do with the presence of the supernumerary polar nucleus. One embryo sac showed four polar nuclei while the synergids were absent (Fig. 44). Here again the probability is that the two nuclei usually destined to develop into synergids had moved down. Another variation in the behaviour of synergids is that occasionally one of them may resemble the egg in appearance (Fig. 47; embryo sac on right).

One abnormal embryo sac showed the full complement of three cells in the egg apparatus and three nuclei in the position of the polars, of which one was slightly larger than the other two. Since only two antipodals could be seen in this case, it is possible that one was behaving as an additional polar nucleus. However, all the antipodals are not always distinguishable as already stated. That it is not an antipodal which is concerned with the appearance of a supernumerary polar nucleus is suggested by a different case. Here the embryo sac possessed, besides egg apparatus of three cells and three antipodals, five free nuclei in the position of the polars (Fig. 45). One of these was larger than the other four. It is possible that the supernumerary nuclei in these two cases do not belong to the embryo sac, for it is known that sometimes nuclei of the surrounding cells may enter the sac. More than the usual number of nuclei may also be seen in a common cavity as a result of fusion of twin sacs which are, indeed, found quite frequently in this plant. It is also possible that one or both of the original polar nuclei might have divided but this is a mere guess.

One mature embryo sac with a three-celled egg apparatus and two polars contained a single antipodal cell which was extraordinarily large in size. The embryo sac thus seemed to be only 6-nucleate. This may either be due to a lack of distinction between two of the antipodals and the surrounding cells, or to early disappearance of the two cells and enlargement of the third, or due to a lack of division of the two chalazal nuclei of the 4-nucleate stage.

Another ovule was exceptional in being hemianatropous and had its embryo sac directed not towards the placenta but in the opposite direction, towards the wall of the ovary.

The variation in the behaviour of the synergids associated with an increase in the number of polar nuclei observed in *Utricularia flexuosa* has not been reported in any other member of the family Lentibulariaceae. Even outside the family it is extremely uncommon. One such case has been reported in *Rudbeckia laciniata* by Battaglia (1947) who observed in the embryo sac " One synergid, one egg and one supernumerary proendospermatic cell, or two eggs and a supernumerary proendospermatic cell " (Battaglia, 1951). Egg-like synergids are also not common and have not been reported in the Lentibulariaceae although known in other families. Johri (1936) has reported synergids in *Sagittaria graminea* which not only look like eggs but also

become fertilized and begin to develop into embryos.

The occurrence of more than eight nuclei in a normally 8-nucleate embryo sac as a result of division of some of the nuclei of the sac is rather rare. Occasional occurrence of 3-5 additional nuclei and their fusion with the polar nuclei has been reported in *Nicotiana* (Goodspeed, 1947; see Maheshwari, 1950) and forms the nearest approach to the 11-nucleate embryo sac in *Utricularia flexuosa* in which five nuclei have been seen in the position of the polars. 6-nucleate embryo sacs produced by a lack of division of the two chalazal nuclei of the 4-nucleate stage are known in several angiosperms.

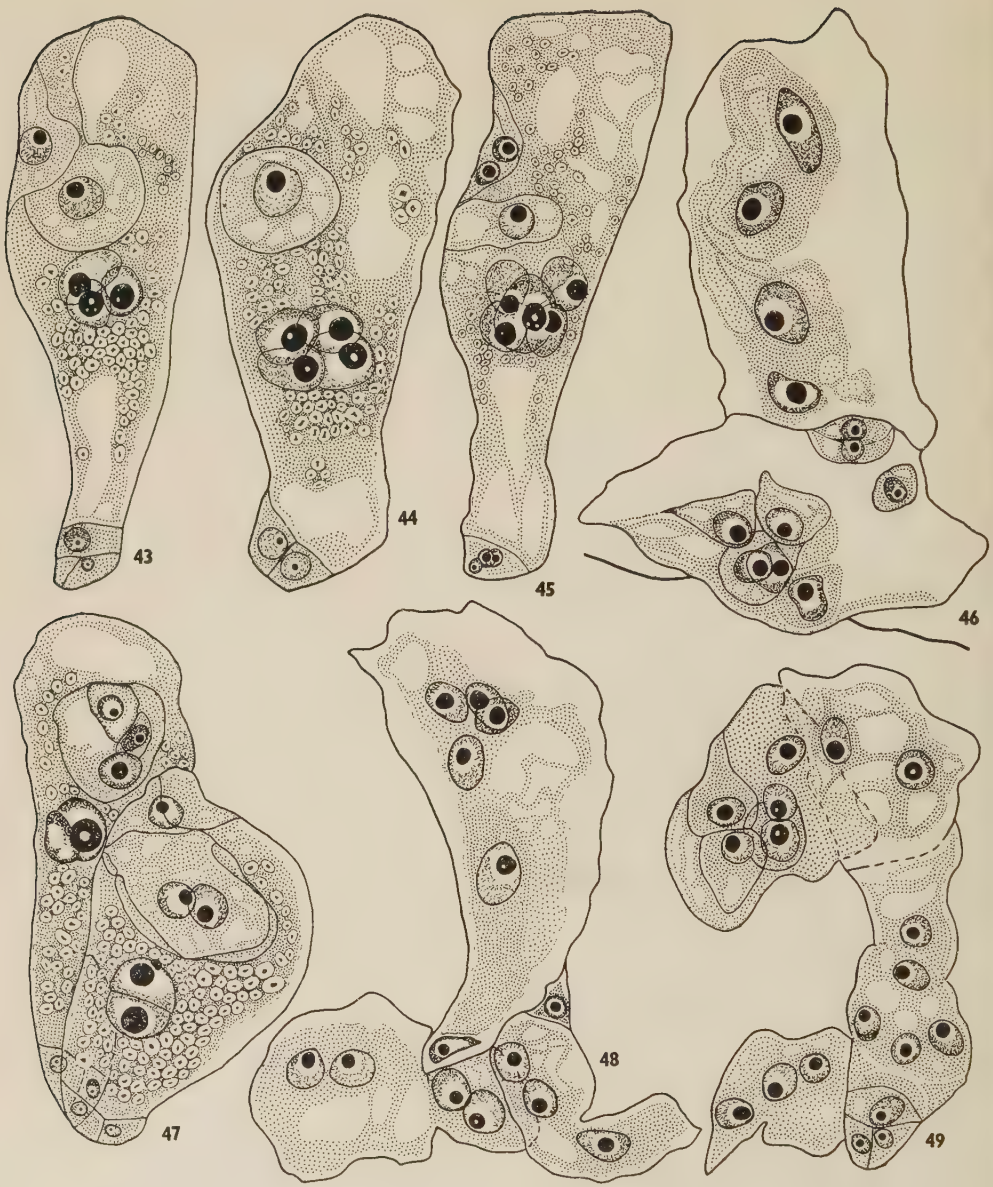
Embryo Sac Showing Reversion of Polarity — At the 4-nucleate stage the embryo sac usually has a narrow chalazal portion and an upper bulbous part which is naked and is in contact with the placenta. In one case although the embryo sac had attained the 4-nucleate stage, its upper end was still covered with the degenerate remains of the nucellar cells. It had remained small and narrow and was still in its original position, inside the ovule, showing no tendency to grow towards the placenta. On the other hand, the chalazal end had become extraordinarily enlarged and curved and had advanced into the funicle to such an extent that it was now situated at a higher level than the original upper end of the sac. This indicates that in exceptional cases the embryo sac may approach the placental nutritive tissue through the funicle instead of following the usual course. Such reversion of polarity seen at the 4-nucleate stage might continue up to the mature stage of the embryo sac so that the egg apparatus is organized in the morphologically lower or chalazal end of the sac (see also Khan, 1953).

In plants belonging to the Balanophoraceae and Viscoideae (Fagerlind, 1945a, 1945b; Rutishauser, 1935, 1937; Steindl, 1935; Schaeppi and Steindl, 1945) the originally lower end of the embryo sac later becomes the upper end, in a manner more or less similar to that described above. In these plants, however, it has been interpreted that the inversion is only apparent rather than true (Oehler, 1927) (see Maheshwari, 1950).

Twin Embryo Sacs, Triplets and Quadruplets — The expectation raised by the frequent occurrence of supernumerary functional megaspores and occasionally mother cells or archesporial cells has been amply fulfilled. One section of a young ovule was seen to contain two healthy megaspores and a degenerated mass situated in a linear manner, constituting obviously a megaspore tetrad. The next section of the same ovule showed a young 2-nucleate embryo sac and a small degenerated mass. It is almost certain that two megaspore tetrads had developed in this ovule.

One ovule contained two young embryo sacs situated one above the other separated by what seemed to be the degenerate remains of the megaspores. The upper embryo sac was 2-nucleate and the lower 4-nucleate. In two cases of twin sacs, the upper sac was oriented as usual while the lower was lying horizontally. The lower side of the lower sac was in contact with the epidermis of the ovule on the chalazal side. A portion of the epidermis had disappeared and the lower sac was in direct communication with the ovary cavity. In one of these cases the lower sac had become 8-nucleate although its organization was irregular (Fig. 46). In two ovules the twin sacs were mature and situated side by side (Fig. 47). In case of the sacs situated one above the other it may be supposed that they arose from two megaspores of a linear tetrad. Their origin from two different mother cells cannot be ruled out altogether although it seems less likely. The twin sacs arranged side by side may have developed from two megaspores situated side by side in a T-shaped or isobilateral tetrad; or from any other megaspores of a tetrad, their side-by-side disposition having been brought about by sliding growth.

Three ovules were found to contain three embryo sacs each. In one case the sacs were arranged one above the other in a linear row and may have developed from the megaspores of the same tetrad. The partition between the



FIGS. 43-49 — *Utricularia flexuosa*. Fig. 43. Abnormal embryo sac with three polar nuclei and only one synergid (reconstructed). Fig. 44. Abnormal embryo sac with four polar nuclei and no synergid at all (reconstructed). Fig. 45. Abnormal 11-nucleate embryo sac with five polar nuclei (reconstructed). Fig. 46. Two embryo sacs in the same ovule. The lower side of the lower sac, which is 8-nucleate and shows unusual organization, has become partly exposed. The line on each side, at the bottom, indicates the limit of the ovule (reconstructed). Fig. 47. Twin embryo sacs both of which are mature. A synergid in the embryo sac on the right has become egg-like (reconstructed). Fig. 48. Three embryo sacs in the same ovule. The fourth megaspore, which is still healthy, is also seen (reconstructed). Fig. 49. Four embryo sacs in a single ovule (reconstructed). × 766.

two lowest sacs was incomplete and very thin. In a case like this the contents of two sacs can easily become incorporated into one and produce embryo sacs with more than the usual number of nuclei. The uppermost sac was the largest and had four nuclei. The other two had three nuclei each. The nuclei of the uppermost sac were largest and those of the lowest the smallest. In the second case of triplets, there were two sacs at the top, side by side, with a third situated below them. Each of the two upper sacs had two large and prominent nuclei while the lower had four nuclei. These triplets might have had developed from a T-shaped or isobilateral tetrad. The third ovule showed one large sac, at the top in the upright position and two smaller ones below it lying side by side. The upper sac contained six nuclei. Of the lower sacs, one had four nuclei and the other three. These figures may be incomplete because the embryo sacs were seen in the first three sections on the slide and it is likely that there might have been some nuclei in the preceding sections which were unfortunately not included in the preparation. In addition to the three embryo sacs, the ovule had a cell which may be regarded as the fourth megaspore of the tetrad (Fig. 48). The three embryo sacs together with the fourth megaspore, may all have originated from an isobilateral tetrad.

The most remarkable case was that of an ovule showing as many as four embryo sacs (Fig. 49). One of them was 2-nucleate and another 3-nucleate. One of the sacs had become 8-nucleate. The fourth sac, which was seen in the last section on the slide, had two free nuclei and three cells that appeared to constitute an egg apparatus. Most probably this sac had also become 8-nucleate and the antipodals had been lost in the sections that were not included in the preparation. The four embryo sacs might have developed from a T-shaped or isobilateral tetrad.

Twin embryo sacs, triplets or quadruplets have not been reported earlier in any member of the Lentibulariaceae. Even *Utricularia vulgaris americana* (Wylie and Yocom, 1923), of which " hundreds of ovaries " were sectioned by the investigators, is not reported to show this phenomenon.

Microsporangium

The anther primordium becomes 4-lobed. Longitudinal sections cut at right angles to the face of the anther show only two lobes while longitudinal sections cut parallel to the face exhibit all the four lobes (Figs. 50, 51). The hypodermal archesporium consists, in each lobe, of a plate of cells which is only one cell thick (Fig. 52). The next stage shows a layer of the primary parietal cells separating the sporogenous layer from the epidermis (Fig. 53). The sporogenous tissue continues to remain one-layered and the cells become the microspore mother cells. Older stages show three layers of cells situated between the epidermis and the mother cells (Fig. 54). The cells of the innermost layer are more or less isodiametric and are destined to form the tapetum. The cells of the other two layers are rectangular and elongated tangentially. The outer of these develops into the endothecium and the inner becomes the middle layer.

The mother cells develop a new wall inside their original wall. The new wall is quite conspicuous and is thicker at the angles (Fig. 55). The mother cells now undergo meiosis (Fig. 56). All the mother cells in an anther and sometimes even in the same chamber do not divide simultaneously. Cytokinesis is simultaneous and takes place by means of cell plates and furrowing (Figs. 57, 58). Later, the microspores acquire their own separate walls although they continue to lie for some time inside the common wall which originally belonged to the mother cell (Fig. 61). The tetrads are mostly tetrahedral (Fig. 58). Decussate tetrads are not uncommon (Fig. 59). Occasionally the microspores may exhibit rhomboid arrangement (Fig. 60). In *U. stellaris* var. *inflexa* both tetrahedral and isobilateral tetrads have been seen. The former type represents the usual condition.

The tapetal cells enlarge and become binucleate but they are not very cons-

picuous. The cells of the middle layer become very narrow. The cells of the endothecium enlarge considerably and develop the characteristic fibrillar thickenings. The tapetum disappears in the mature anther, the endothecium becomes very prominent while the cells of the epidermis and the middle layer exhibit various degrees in their disintegration (Figs. 55, 70, 71).

The development of the anther and microsporogenesis in the Lentibulariaceae have been ignored by earlier workers and no information is available for comparison except on *U. coerulea* (Kausik, 1938) which resembles *U. flexuosa* in these respects.

Male Gametophyte

Pollen Morphology — The exine of the pollen grain is thick but transparent. According to the pattern on the exine the pollen is divisible into two types. In one type, which is much more common than the other, the exine consists of broad, longitudinal strips running from pole to pole (Fig. 62). They are broader in the middle and taper towards the poles where the ends of some of them may meet with one another. The strips or bands alternate with narrow furrows. The number of the strips usually varies between 14 and 19. At the equator the bands are seen to bulge out rather conspicuously. In other parts they are almost flat or only slightly curved. As a consequence of this, sections of grains, passing partly through the equatorial region and partly through the extra-equatorial region, show the exine consisting of prominent bulges in one part and of almost flat or slightly curved bands in another part (Figs. 65, 66). In older grains the prominence of the equatorial bulges decreases. The grains exhibit a flattening at the poles. The diameter of the grain as seen in polar view measures about 35 μ in glycerin jelly mounts. Upon the stigma the grains often measure much more; the diameter in polar view may be up to about 42 μ and in equatorial view about 30 μ.

In the second type the essential pattern is the same but the surface of the grain is divided into four areas disposed tetrahedrally (Figs. 63, 64). The centre of each area acts as a pole so that here there are four poles instead of two of the first type. Strips of exine starting from the centre of one area pass into the three adjacent areas and approach their centres. Or, in other words, strips originating at three different centres converge towards the fourth. In this type the boundary lines separating the four areas from one another are analogous to the equator of the first type because the strips exhibit prominent bulges along these lines. The disposition of the four areas may not always be exactly tetrahedral but may sometimes show a slight variation. It seems as if these grains had been produced as a result of non-separation of the four microspores of a tetrad. Since the pattern in this type is more complicated, sections of grains show the exine as more irregular in this type than in the first type. These grains do not differ much in size as compared with those of the first type. The two types of grains may occur in the same flower but usually one type predominates in any individual flower, the grains of the other type being few in number.

The structure of the exine of the first type of pollen grains in *U. flexuosa* resembles that in *U. intermedia*, *U. minor*, *U. neglecta* and *U. vulgaris* which possess about 15 to 20 furrows (Fischer, 1890, cited by Erdtman, 1943). *U. vulgaris*

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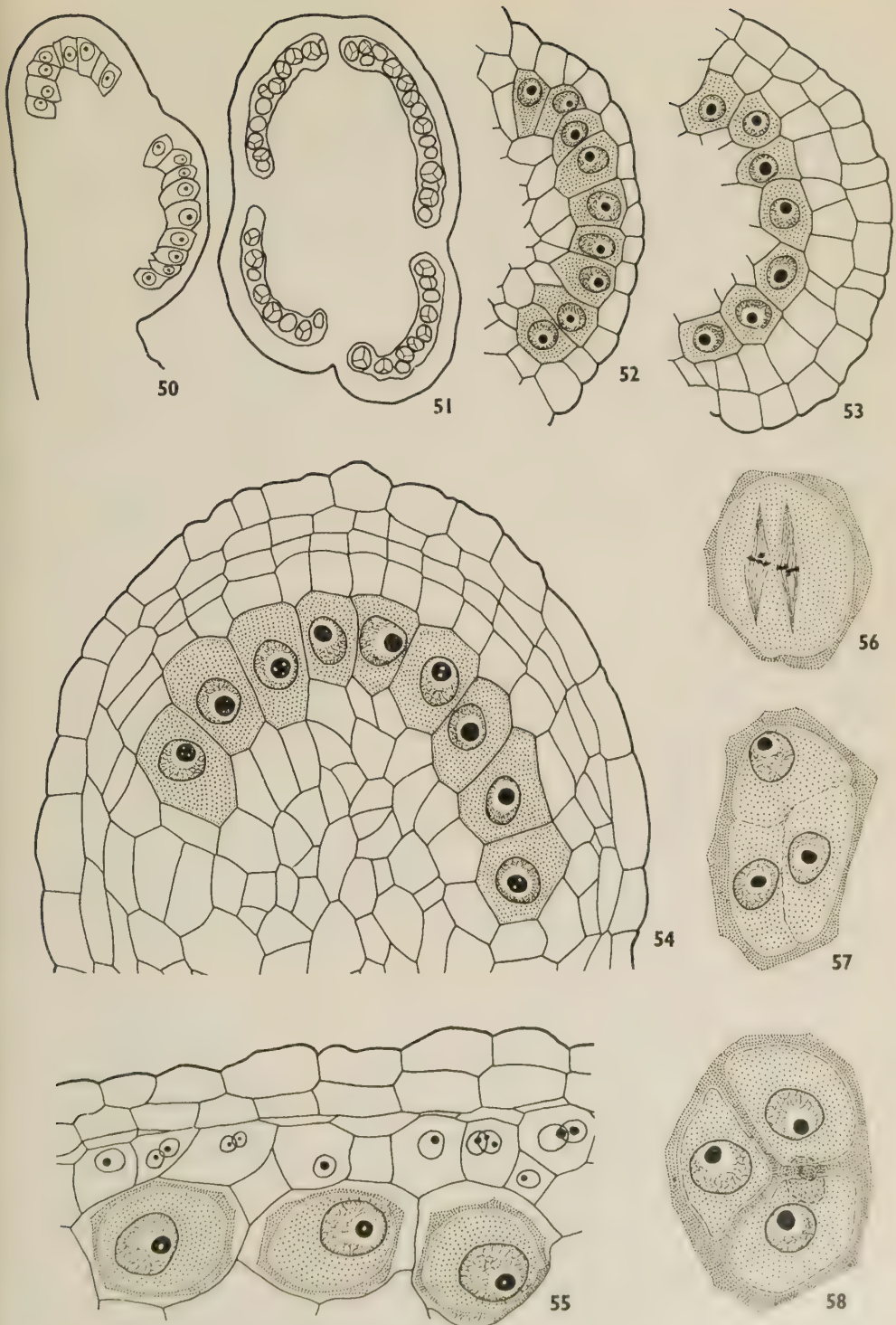
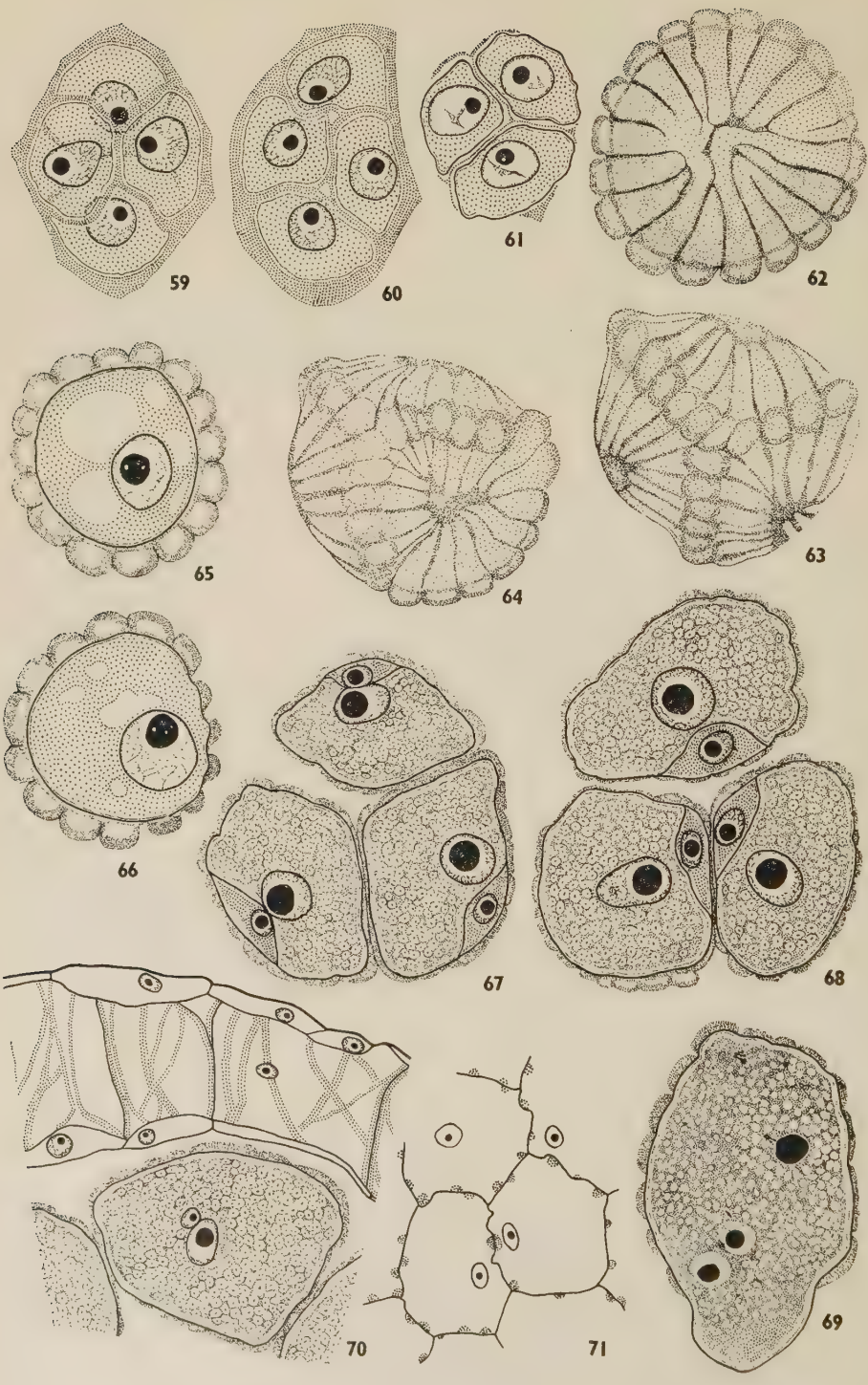
Figs. 50-58 — *Utricularia flexuosa*. Fig. 50. A young stamen as seen in l.s. cut at right angles to the face. Fig. 51. L.s. of anther cut in a plane parallel to the face, showing all the four chambers. Fig. 52. Part of anther showing hypodermal archesporium. Fig. 53. The sporogenous tissue and the primary parietal layer. Fig. 54. Older stage in anther development, showing microspore mother cells, tapetum, middle layer and the layer of cells (situated next to epidermis) that would develop into endothecium. Fig. 55. Microspore mother cells with new walls. Some of the tapetal cells have become binucleate. Fig. 56. Microspore mother cell showing meiosis. Fig. 57. Cytokinesis of the microspore mother cell. Fig. 58. Tetrahedral tetrad of microspores. Fig. 50. × 490. Fig. 51. × 132. Figs. 52-55. × 918. Figs. 56-58. × 1341.



FIGS. 50-58.



FIGS. 59-71.

americana (Wylie & Yocom, 1923) is also similar. In *U. coerulea* (Kausik, 1938), on the other hand, the exine has been described as thick but smooth. The figures show only three furrows. The pattern described for the second type of grains in *U. flexuosa* has not been reported in any other species of *Utricularia*. The pollen grains of *U. vulgaris* measure 45 μ by 36 μ according to Erdtman (1943) and of *U. vulgaris americana* 26 μ × 30 μ according to Wylie and Yocom (1923). The measurements of the grains of *U. flexuosa* are, therefore, intermediate between those of *U. vulgaris* and *U. vulgaris americana*.

Development of Male Gametophyte — The young microspore has dense cytoplasm with a large nucleus (Fig. 61). Prominent vacuoles develop in the cytoplasm later, but disappear by the time the pollen grains become binucleate (Figs. 65-68). The generative cell is small and is situated, at first, on one side, along the wall of the pollen grain. Its cytoplasm is, in many cases, seen to be distinctly separated from the remaining cytoplasm of the grain by a clear space which may be taken to correspond to the wall separating the generative cell from the original cell. The position in which the generative cell is formed with reference to the outer or the inner side of the pollen grain could not be determined because at this stage the grains are no longer associated in tetrads. In a few cases the grains were seen to be so arranged that they appeared to form tetrads. In some of these the generative cell was seen towards the outer side while in others it was situated towards the inner side. Most probably these cases represent chance groupings of grains and are to be regarded as without any significance (Figs. 67, 68). They should serve as a warning against hasty conclusion regarding variation in position in which the generative cell is formed in the same species.

With the advent of the 2-nucleate condition the pollen grains become packed with starch grains (Figs. 67, 68). The starch grains may be stained light pink with safranin or may be almost colourless. The hilum appears as a shining body. If the grains remain colourless and if the hilum also is not distinctly visible, they may give the impression of being vacuoles. The pollen grains become 3-nucleate before they are shed from the anthers (Fig. 69).

The male gametophyte of *U. stellaris* var. *inflexa* was studied in acetocarmin preparations. The mature pollen was 3-nucleate. Most of the grains examined in preparations made from mature anthers had already germinated. This was not expected. Some pollen grains had produced two tubes of which only one had developed to full length. The tubes exhibited curves here and there and the diameter was not uniform throughout the length. Some tubes showed swellings and convolutions while others exhibited a tendency for branching. Some grains had well-developed pollen tubes, but the gametes and the vegetative nucleus were still inside the grain. Pollen grains that had germinated inside the anthers were noted in *U. flexuosa* also, but they were not as frequent in this species as in the other.

The germination of the pollen grains inside the anther has not so far been reported in the family Lentibulariaceae. In other angiosperms this has been reported mostly in cleistogamous flowers (see Maheshwari, 1949). The occurrence of the phenomenon in *Utricularia* in which

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Figs. 59-71 — *Utricularia flexuosa*. Fig. 59. Decussate tetrad of microspores. Fig. 60. Rhomboid tetrad (reconstructed). Fig. 61. Tetrahedral tetrad with the wall of the mother cell breaking up. Fig. 62. Polar view of a pollen grain of the first type (see text). Figs. 63, 64. Pollen grain of the second type (see text) as seen in two different foci. Fig. 65. Microspore showing equatorial bulges of exine all round. Fig. 66. Microspore showing equatorial bulges of exine on one side and the bands of exine cut across, on the other side. Figs. 67, 68. Groups of pollen grains showing generative cell and vegetative nucleus. Note that such groups of grains may be mistaken for tetrads and may lead to wrong conclusions regarding the position in which generative cell is cut off. Fig. 69. Mature 3-nucleate pollen grain. Fig. 70. Part of anther showing cells of endothecium with fibrillar thickenings. The tapetal cells have disappeared. Fig. 71. Cells of endothecium as seen in tangential section of anther. Figs. 59-69. × 1214. Figs. 70, 71. × 831.

the flowers are not cleistogamous, seems, therefore, to be worthy of note. A similar occurrence has recently been reported in *Stylidium graminifolium* (Subramanyam, 1951).

Variation in Development of Male Gametophyte — Pollen grains possessing gigantic proportions have been observed occasionally (Fig. 72). The extraordinarily large size of the nuclei was as impressive as the size of the grain as a whole. The grain may become lobed or develop folds in the wall, probably because of large size. The large size of the nuclei suggests that they may be polyploid.

An interesting feature noted in the anthers of several flowers is the presence of compound pollen grains. They are quite common and consist of two to four cells enclosed within a common exine and separated from one another by thin walls. The cells in a compound grain are similar to one another in their size, in the size of their nuclei and in the presence or absence of starch grains in the cytoplasm. The compound grains are of two kinds. Some grains consist of two to four cells and are characterized by the absence of starch grains. In the 4-celled grains, the arrangement of cells may be tetrahedral, decussate, T-shaped (Fig. 73) or isobilateral (Fig. 74). A comparison of Figs. 73, 74 showing these grains, with Figs. 59-61, 65, 66 showing microspore tetrads and microspores, indicates that these compound grains are equivalent to tetrads in which the microspores have failed to separate and have developed a common exine. This conclusion recalls the second type of pollen grains described earlier. Compound grains of this kind containing less than four cells can be explained as parts of tetrads cut across during sectioning. If this interpretation is correct, the varied arrangement of the four cells in the compound grains would be noteworthy. Many compound pollen grains consist of only two cells enclosed within a common exine and characterized by the presence of a large number of starch grains. The wall separating the two cells may be very faint or incomplete or even absent (Figs. 75-77). In the latter case the pollen grain contains two large nuclei of equal size. It may be that the wall was not formed at all or that it was formed but disappeared later. A comparison of Figs. 75-77 showing these grains, with Figs. 59-61, 65, 66, showing microspore tetrads and microspores, on the one hand, and with Figs. 67, 68, showing male gametophytes at the 2-nucleate stage of development, on the other hand, reveals their marked resemblance to the latter. It seems obvious to conclude that the first division in some microspores produces two cells or nuclei of equal size instead of one small generative cell and the other large vegetative cell. These compound grains may, therefore, be regarded as equivalent to single microspores which are potentially capable of developing into double gametophytes (see also Moffett, 1931-32).

Pollen grains containing supernumerary nuclei or what may be described, in some cases, as double gametophytes in various stages of development, have been observed. In one case the grain contained two pairs of nuclei (Fig. 78). One pair consisted of one large and one small nucleus, the latter being organized as a distinct cell. The difference in size of the nuclei of the other pair was not very appreciable and none of them was organized as a cell. This grain may either be regarded as equivalent to two male gametophytes, each at the 2-nucleate stage, or simply as showing two supernumerary nuclei. Another grain was seen to possess two small and two large nuclei (Fig. 79). The small nuclei were

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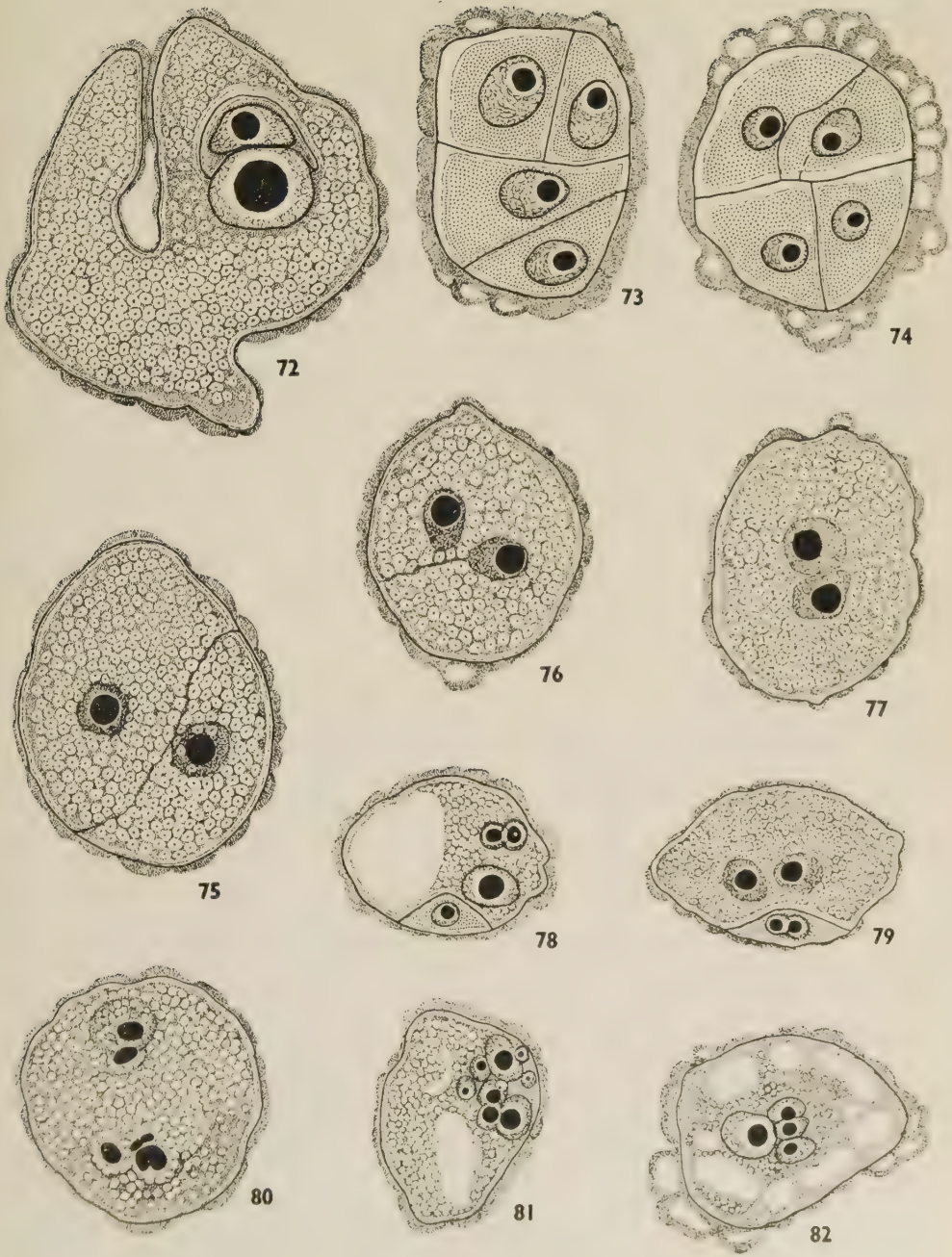
Figs. 72-82 — *Utricularia flexuosa*. Fig. 72. Giant pollen grain with generative cell and vegetative nucleus. Figs. 73, 74. Four-celled compound pollen grains. Fig. 75. Two-celled compound pollen grain. Fig. 76. Pollen grain with two large nuclei of equal size and incomplete partition wall. Fig. 77. Pollen grain with two large nuclei. Fig. 78. Pollen grain with a generative cell, a vegetative nucleus and two supernumerary nuclei. Fig. 79. Pollen grain with two sperms and two large nuclei of equal size. Fig. 80. Pollen grain with two gametophytes. Fig. 81. Pollen grain with six large nuclei and three micronuclei. Fig. 82. Pollen grain with one large nucleus and three small nuclei. × 1341.



FIGS. 72-82.

situated in dense cytoplasm on one side. These, together with one large nucleus, may be regarded as equivalent to one gametophyte. The other large nucleus may be regarded either as a daughter of the original microspore nucleus, potentially capable of producing another gametophyte or as a supernumerary vegetative nucleus. A different grain showed one large and one small nucleus on one side while on the other side were seen three chromatin bodies; about one of the latter it was difficult to say whether it was really one or two (Fig. 80). One pollen grain contained more than half a dozen nuclei placed close together (Fig. 81). One may distinguish, in this case, two sets of three nuclei each, one large and two small, and some micronuclei. The two sets of nuclei may or may not represent two gametophytes. One pollen grain contained one large nucleus and three small ones lying close together in the middle of the grain (Fig. 82). The cytoplasm exhibited conspicuous vacuoles. Other grains in the anther were either 2- or 3-nucleate. It may be a case of polyspermy, the three small nuclei being interpreted as equivalent to sperms. But the cytoplasm is not ordinarily expected to show large vacuoles at this stage.

Very little work has been done on the development of the male gametophyte in Lentibulariaceae except on *U. coerulea* which follows the usual course. The variations described above are, therefore, unknown in the family. Even in the order Tubiflorae they are not common. Some of the features reported here have been described in *Cuscuta* by Fedortschuk (1931).

Course of Pollen Tube — Although there is an open stylar canal, the pollen tubes do not enter it directly. The tubes make their way between the stigmatic papillae and enter the tissue of the style. The cells in the upper region of the stigmatic side of the style are elongated in a longitudinal direction, and thus seem to facilitate the passage of the tubes. The tubes move between the cells and there is no evidence that they destroy any cells during their passage. After having travelled some distance, the tubes turn towards the stylar canal and enter it. Then they move down along the stylar canal ectotropically. On reaching the base of the canal, some of the tubes cross over directly to the placenta while others creep along the inner surface of the ovarian wall for varying distances before they turn towards the placenta (Fig. 83).

As the embryo sac grows beyond the integument and a part of it is situated in the ovary cavity, the pollen tube does not have to enter the ovule at all in order to reach the embryo sac. It approaches the embryo sac through the passage between the integument and the placenta (Fig. 84). Although it comes in contact with the naked embryo sac here, it does not enter it at this point. It proceeds along the apical part of the embryo sac, between its membranous wall and the placenta, to the funicular side, entering the embryo sac in the neighbourhood of the synergids. Why should the pollen tube consistently follow a circuitous path to reach the funicular side and enter the embryo sac in the vicinity of the synergids, and, why should it not enter the sac at the point where it first comes in contact with it are fascinating questions. It may be that, as generally presumed, the synergids exercise a chemotactic influence on the tube. If they really do so, why should they not direct the tube to approach them straightway, by penetrating the embryo sac at the place where it first touches it? Why should they direct the tube to follow a roundabout course? Is it that in their neighbourhood the tube finds a weak spot in the wall of the embryo sac which it does not find elsewhere and which makes its entry into the sac at that particular spot easier than anywhere else?

The course of the pollen tubes in *U. vulgaris americana* (Wylie & Yocom, 1923) closely agrees with the species under study. However, in *U. vulgaris americana* all the pollen tubes approach the placenta directly without some of them creeping along the inner surface of the ovary wall. In *U. coerulea* the pollen tubes are stated to pass down along the conducting cells of the stylar canal. In all species of *Utricularia* studied so far, except *U. purpurea*, the pollen tube is described as entering the embryo sac outside the ovule. There is thus no porogamy in these plants.

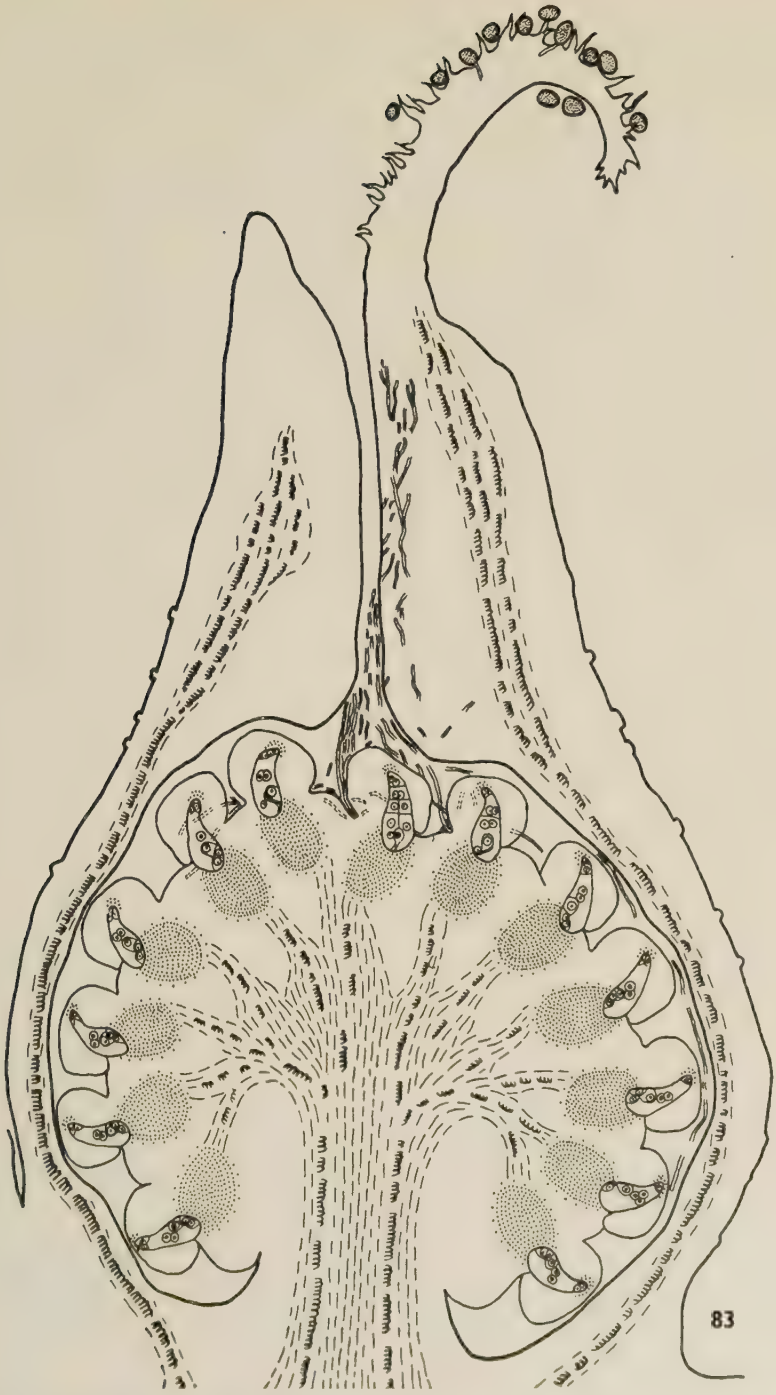


FIG. 83 — *Utricularia flexuosa*. L.S. of gynaeceum showing course of pollen tubes (diagrammatic reconstruction). Note also vascular supply of placenta, placental nutritive tissue and haustorial apices of embryo sacs embedded in placental tissue. × 94.

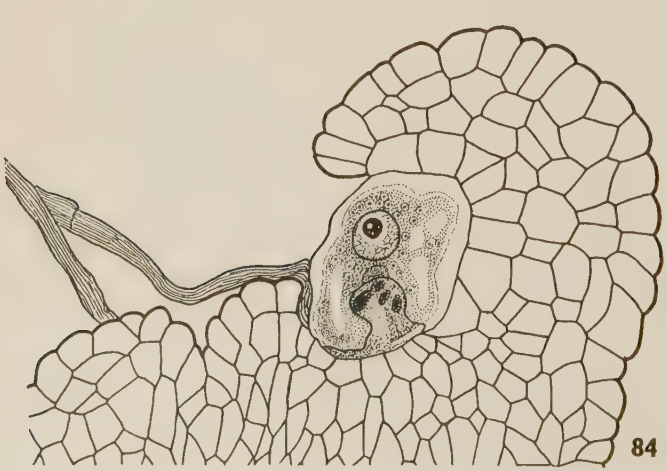
In *U. purpurea* Merz (1897; cited by Schürhoff, 1926) has described the pollen tube as entering the embryo sac either from the side or from the direction of the chalaza. In *Genlisea* and *Pinguicula* the embryo sac remains inside the ovule and porogamy has been reported in the former (Merl, 1915).

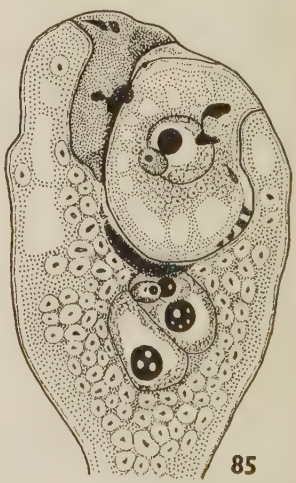
Fertilization

The mature embryo sac ready to receive the pollen tube usually contains both the synergids intact. The polar nuclei lie near each other, but do not fuse. They have been seen to remain unfused even in much older ovules which had failed to receive the pollen tube although other ovules in the same ovary showed advanced stages in development. The embryo sacs containing pollen tube possess only one synergid, the other being presumably destroyed during the entry of the pollen tube. The persistent synergid has been seen till the embryo becomes about four cells wide and ten cells long, excluding the suspensor (Fig. 112).

The contents of the pollen tube inside the embryo sac usually include certain structures which vary in number, shape and size. They are stained bright red with safranin and often constitute a conspicuous feature of the embryo sac (Figs. 85-88). They may be compared to the X-bodies described in some plants (see Maheshwari, 1950). The end of the pollen tube, in one embryo sac, seemed to have spread into a small cap fitting upon the egg. In another case it was seen pressing against the egg and had produced a depression in it. In one embryo sac the end of the tube had penetrated into the egg. Double fertilization has been observed (Fig. 85). One male gamete was seen near the egg nucleus and the other near the two polar nuclei. The polar nuclei were in intimate contact with each other and their adjacent walls had become flattened due to mutual pressure, but they had not fused yet. Near the polars and the second male gamete lay another nucleus which might have belonged to the persistent synergid and may have become displaced from its original position. No synergid was seen in this case in the usual position, although post-fertilization embryo sacs usually show one persistent synergid (cf. occurrence of three polar nuclei described earlier).

In *U. coerulea* (Kausik, 1938) one of the synergids is destroyed during the





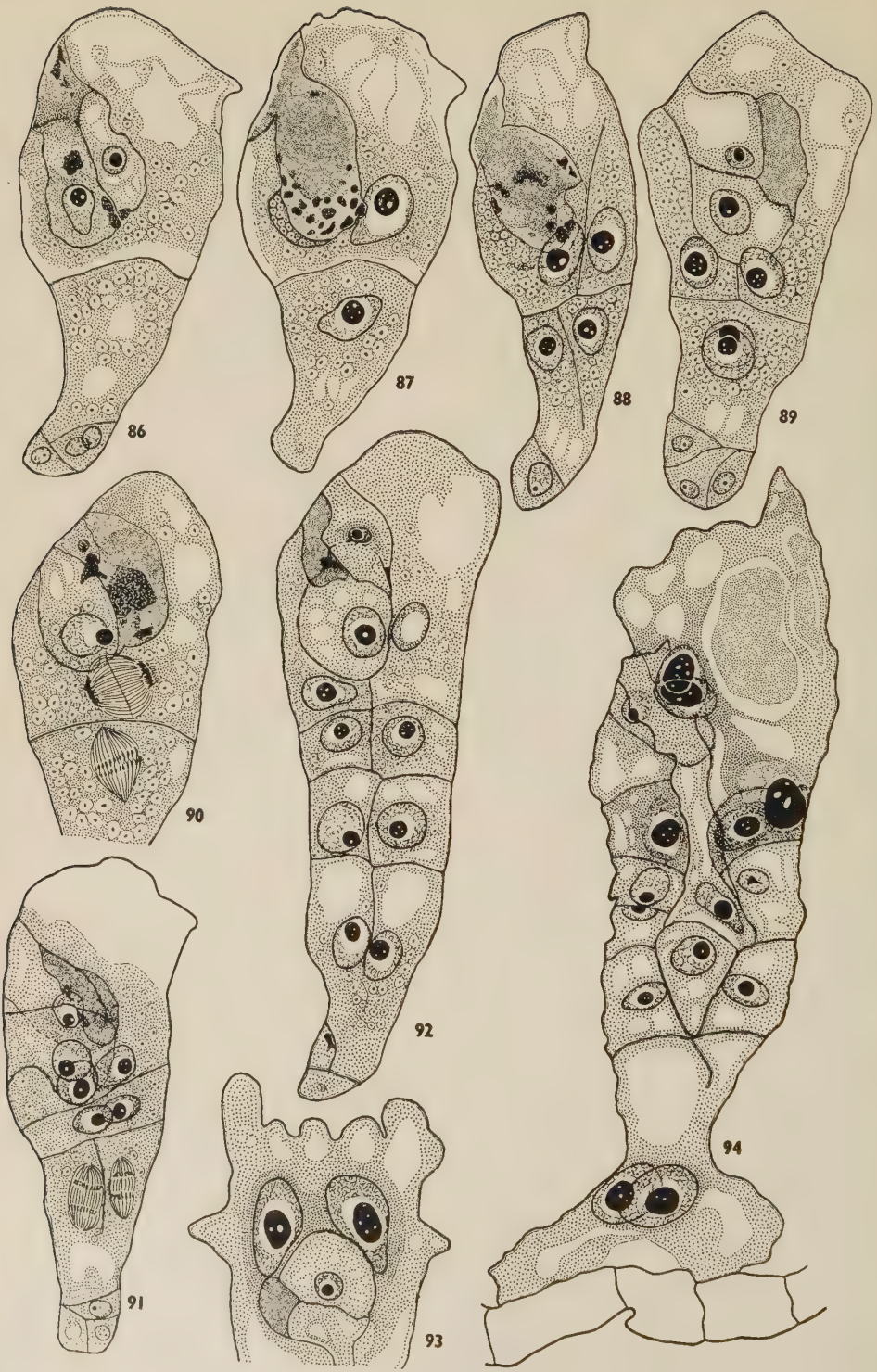
FIGS. 84, 85 — *Utricularia flexuosa*. Fig. 84. The pollen tube as it approaches and enters the embryo sac (reconstructed). Fig. 85. Embryo sac showing double fertilization. The two polar nuclei are still unfused. Near the polars and the second male gamete is seen another nucleus which may belong to the persistent synergid. Note dark bodies in the pollen tube and around the egg. Fig. 84. × 542. Fig. 85. × 1017.

entry of the pollen tube as in *U. flexuosa*. A study of the illustrations of *U. vulgaris americana* (Wylie & Yocom, 1923) shows that a synergid is destroyed in this species also. Double fertilization has been observed in *U. vulgaris americana* and *U. coerulea*. In both the species, as in *U. flexuosa*, the fusion of the polar nuclei is very much delayed.

Endosperm

The development of the endosperm begins with the division of the primary endosperm nucleus and a transverse partitioning of the entire embryo sac into two chambers. The primary micropylar chamber contains, in addition to its own nucleus, the zygote, the persistent synergid and the remains of the pollen tube and presumably of the degenerated synergid. The primary chalazal chamber contains its own nucleus and the antipodals (Figs. 86, 87). Each of the two primary chambers usually divides by a longitudinal wall. The walls do not extend completely towards the extremities. The walls in the two chambers may be disposed so as to produce either a plate of four cells or the four cells may be arranged decussately (Figs. 88, 89). Occasionally the nuclear spindle in one chamber is oblique with reference to that in the other. In one case the orientation of the spindles was such that the resulting four cells would have shown a T-shaped disposition (Fig. 90). The division of the two cells may not always be simultaneous so that a 3-celled stage may occasionally be seen. It is in the chalazal chamber that the division lags behind (Figs. 90, 91). Fig. 91 shows a stage after the 4-celled condition. The upper two cells have become divided transversely into four cells while the lower two are in the process of division. After the completion of this division, the endosperm consists of 8 cells arranged in four tiers of two cells each. All the eight cells may be arranged in the same plane so that they form a plate or the upper four cells may be in one plane and the lower four in a plane at right angles to that of the upper. This results from a decussate arrangement at the 4-celled stage. The partition wall separating the two cells in the two terminal pairs remains incomplete; in older stages it is usually not seen. The endosperm is now divisible into three distinct parts (Fig. 92): (1) The micropylar end consists of two incompletely separated cells or one binucleate chamber and develops into the micropylar haustorium. The zygote which is still undivided, the persistent synergid and the remains of the pollen tube are seen in this haustorium. (2) The chalazal end consists similarly of two incompletely separated cells or one binucleate chamber and develops into the chalazal haustorium. (3) The middle portion consists of four cells which produce the endosperm proper. Since two of these cells have been derived from the primary chalazal chamber, this chamber plays as important a role in the development of endosperm proper as the primary micropylar chamber.

The micropylar haustorium soon shows its aggressive character. Its apical part is situated in the placental nutritive tissue from the very beginning because it is formed in the apex of the embryo sac. This haustorium thus takes up a function that had already been initiated by the embryo sac. Its nuclei move up and its surface develops a number of small bulbous lobes (Fig. 93). These add to the haustorial surface and penetrate deeper into the nutritive tissue, coming in touch with an increasingly larger number of cells. The cytoplasm of the haustorium is dense and its two nuclei become exceptionally large and conspicuous. The number of haustorial nuclei increases later at two different stages in two different ways. The first of these is a novel feature of this plant and has been noted in several cases. When the endosperm is still comparatively young, the contents of those cells of the endosperm proper which are adjacent to the micropylar haustorium acquire a marked resemblance to those of the haustorium. Their cytoplasm becomes dense and the nuclei become large. The walls of some of these cells break down and the contents become incorporated into the haustorium. As a consequence, the haustorium comes to possess more than two nuclei (Fig. 94). Other cells of the endosperm proper,



FIGS. 86-94.

whose contents have acquired resemblance to those of the haustorium, may remain intact. They also probably assist the haustorium in its function and it may, therefore, be said that sometimes the haustorium also becomes multicellular (Fig. 94). (See, however, Crété, 1951, for a definition of an endosperm haustorium.)

The micropylar haustorium expands and extends further and further into the placental nutritive tissue, breaking up the cells that come in contact with it. The cells in the immediate neighbourhood of the haustorium appear as if they are dissolving or melting away. Nuclei of many cells of the placental nutritive tissue that have already been disorganized are seen in the cytoplasm of the haustorium (Fig. 95). As the haustorium enlarges, the numerous little lobes that had appeared earlier are obliterated and the entire haustorium becomes one large bulb-like structure (Figs. 95, 96). With further increase in size, the number of placental nuclei in the haustorium increases. At about a stage shown in Fig. 96 the number of these nuclei is nearly a hundred. At an older stage, such as that shown in Fig. 97, the number exceeds one hundred and in some cases more than 150 nuclei have been counted. It is at about this advanced stage that the second method of increase in the number of haustorial nuclei is seen to operate. The original nuclei of the haustorium are seen to have become very large and to have assumed irregular shapes even at an earlier stage (Figs. 95, 96). It seems that later they break up into a number of separate lobes which then form independent nuclei (Fig. 97).

A variation in the behaviour of the micropylar haustorium was noted in one preparation. It consisted in the development of a lateral lobe which was protruding out through the passage between the integument and the placenta. It had touched the back of a neighbouring ovule, but had caused no damage to it (Fig. 98).

The chalazal haustorium is also very aggressive. It enlarges and destroys the cells in its vicinity, eventually coming in contact with the epidermis of the ovule. It then begins to expand and acquires a peltate or bulbous form (Figs. 94, 95). The cells of the epidermis with which the haustorium comes in contact seem to remain immune from its influence for some time, but eventually they also succumb. As they become weaker, the outline of the ovule in this region shows a distortion, and, in older stages, the epidermis may become ruptured. The number of nuclei in the chalazal haustorium usually remains two. Only occasionally it may have three nuclei (Fig. 98). The origin of the supernumerary nucleus could not be determined. Although many chalazal cells are destroyed by the haustorium, their nuclei are not seen to enter it. Occasionally a few small nucleoli are seen in the haustorium. It was at first thought that they belonged to the nuclei of the neighbouring cells. It seems, however, more probable that they belong to nuclei of the haustorium itself.

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FIGS. 86-94 — *Utricularia flexuosa*. Figs. 86, 87. Consecutive sections of an embryo sac showing the primary micropylar and chalazal chambers formed after the first division of the primary endosperm nucleus. Note the fertilized egg (Fig. 86), persistent synergid (Fig. 86), pollen tube and dark bodies in the micropylar chamber, and, antipodals in chalazal chamber (Fig. 86). Fig. 88. 4-celled endosperm with the cells arranged in one plane. Fig. 89. 4-celled endosperm with decussate arrangement of cells (reconstructed). Fig. 90. Nuclei of the two primary chambers in division. The resulting four cells would exhibit a T-shaped disposition (reconstructed). Fig. 91. The two micropylar cells of the 4-celled endosperm have divided transversely into four cells. The two chalazal cells are dividing (reconstructed). Fig. 92. 8-celled endosperm with the micropylar and chalazal haustoria and four initials of the endosperm proper (reconstructed). Fig. 93. The 2-nucleate micropylar haustorium with persistent synergid and part of zygotic tube. Note the lobes of haustorium (reconstructed). Fig. 94. Older endosperm. Note the cells adjacent to the micropylar haustorium. The contents of one cell have become incorporated into the haustorium which is now 3-nucleate. The chalazal haustorium has reached the epidermis of the ovule. The persistent synergid is seen in the micropylar haustorium at the base of the 2-celled proembryo (reconstructed). Figs. 86-93. × 762. Fig. 94. × 728.

An interesting variation in the mode of endosperm development has been noted in one ovule in which the endosperm consisted of seven cells situated one above another in a linear row (Fig. 99).

In the sequence of early cell divisions and the mode of differentiation of haustoria, *U. flexuosa* resembles *U. coerulea*, both the species conforming to the Scutellaria type (Schnarf, 1917). In the organization of the mature endosperm and the behaviour of the haustoria *U. flexuosa* resembles *U. vulgaris americana*. In the manner in which the micropylar haustorium sometimes becomes multinucleate and multicellular in earlier stages, it differs from both and presents a distinctive feature. An exact parallel, as far as I am aware, has not been reported earlier in other plants although, in the mode of addition of cells from the endosperm proper to the haustorium, a similarity can be noted with some plants (see Junell, 1934; Pal, 1951; Crété, 1951).

Both micropylar and chalazal haustoria have been reported in *Polypompholyx* (Lang, 1901) and *Genlisea* (Merl, 1915). The early stages in endosperm development in the Lentibulariaceae, however, are not known satisfactorily. Summarizing the information available, one may distinguish three modes of development. The first is seen in *Pinguicula* in which the 8-celled stage is derived in conformity with the Scutellaria type. In *P. alpina* no haustorium, either micropylar or chalazal, is present. In *P. vulgaris* a 2-celled chalazal haustorium is present, but the micropylar haustorium does not develop or may be said to be very feebly developed. The second mode of development is seen in *U. coerulea* and *U. flexuosa* both of which conform to the Scutellaria type and also possess well-developed micropylar and chalazal haustoria. The third mode, which seems to be doubtful, is presumed to occur in *U. vulgaris americana* (Wylie & Yocom, 1923) and has been compared by Schnarf (1931) with the condition in some Rhinanthoideae. The endosperm which Wylie and Yocom saw in this species, after the first division of the embryo sac into the two primary chambers, was fairly old and had the two haustoria and the endosperm proper already well differentiated. They did not see the second and third divisions of the primary endosperm cell. A comparative study of the morphological and embryological characters shows that, on the whole, *U. vulgaris* and *U. flexuosa* resemble each other more closely than *U. coerulea*. On the contrary, in the mode of endosperm development, *U. vulgaris* is supposed to be distinctly different from *U. flexuosa*. This difference between the two species, therefore, although not impossible, does not seem to harmonize with the marked resemblances in other features. This becomes all the more remarkable when the question is considered in the light of the fact that *U. flexuosa* and *U. coerulea*, so different in other respects, show the same mode of development. Even *Pinguicula*, a different genus, conforms to the same Scutellaria type.

In marked contrast to the uniformity in embryo sac development in the order Tubiflorae is the great variety in the mode of endosperm development. All the three major types, namely nuclear, cellular and helobial are represented in the order and each of them exhibits its own variation (see Schnarf, 1931). The extraovular endosperm haustorium occurs in the Tubiflorae, besides Lentibulariaceae, in *Galeopsis* of the Labiatae (Schnarf, 1917) and *Globularia* of the Globulariaceae (Billings, 1901; Rosén, 1940; see Schnarf, 1931 and Maheshwari, 1950). In these

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FIG. 95 — *Utricularia flexuosa*. L.S. of an old ovule showing endosperm and embryo. A part of placenta is included to show the nutritive tissue. The micropylar haustorium has broken down some cells of this tissue and their nuclei are seen in the haustorium. The two nuclei of the haustorium have become irregular in shape. The embryo is embedded in cellular endosperm, but the suspensor extends into the micropylar haustorium. The persistent synergid is seen near the base of suspensor. Note the beginning of differentiation in the cells of endosperm proper near the extremities (reconstructed). × 409.

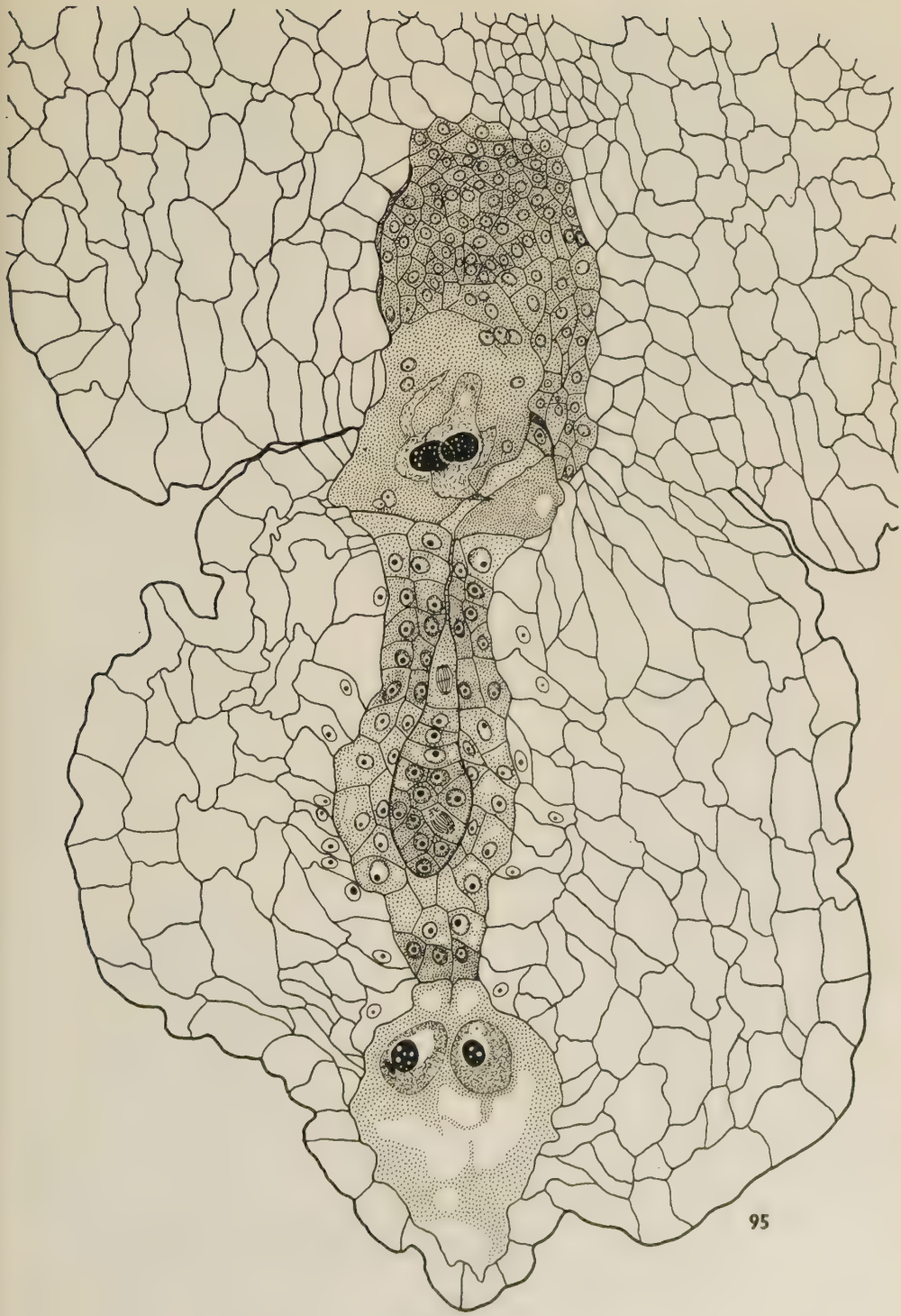
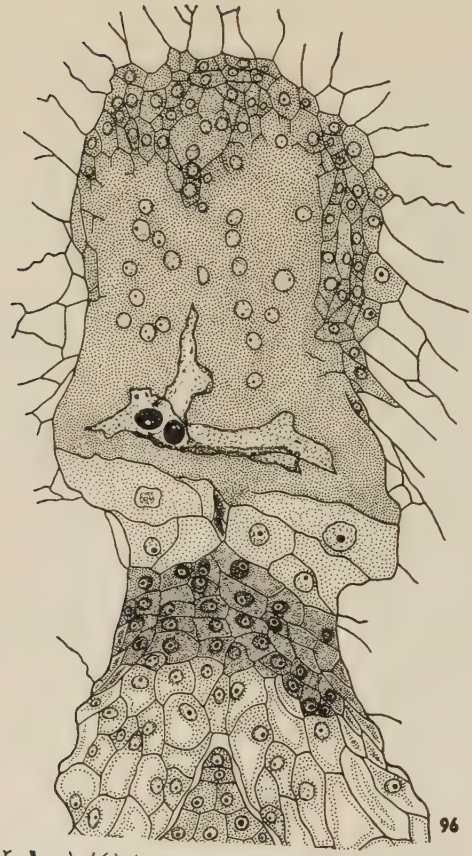
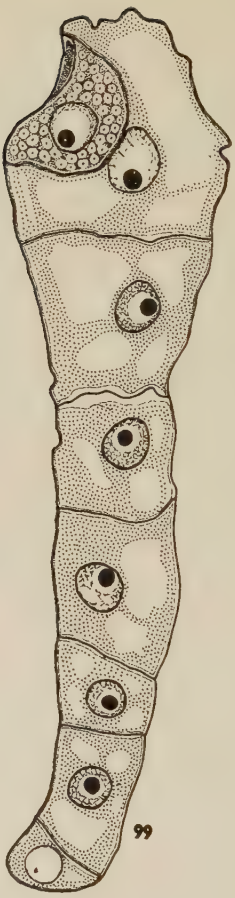
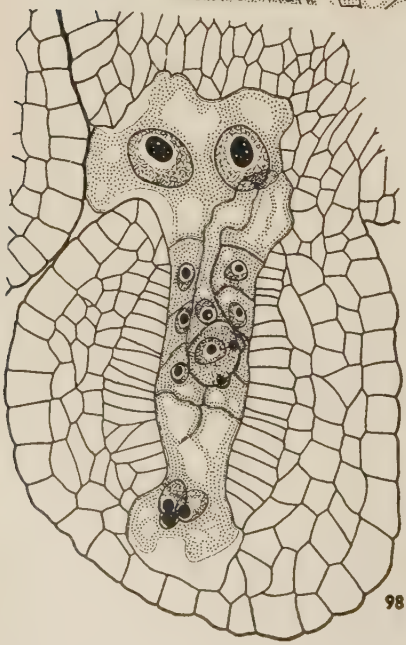
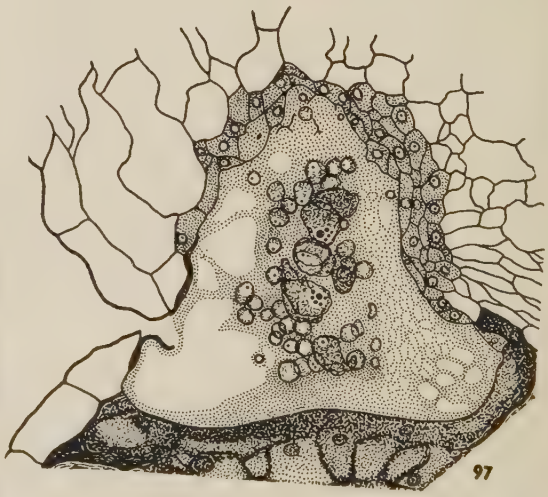


FIG. 95.









FIGS. 96-99.

the haustoria may extend as far as the wall of the ovary.

Embryo

The zygote divides much after the primary endosperm nucleus. The persistent synergid is often seen in such a position in relation to the zygote that the two together seem to form a single 2-celled structure and one might be misled to suppose that the zygote has divided. A comparison with prefertilization embryo sacs reveals the true nature of the two cells. The first sign of embryo development is the appearance of a protuberance at the apical end of the zygote. The protuberance grows into a long tube into which the nucleus of the zygote migrates (Figs. 100-102). As the zygotic tube elongates, it penetrates into the cellular endosperm, its tip reaching very near the chalazal haustorium. The nucleus is situated near the apex. The cell divisions occur in the tip of the zygotic tube and the resulting embryo is placed in the midst of the cellular endosperm which surrounds it almost completely. The only part that is not surrounded by it is the basal part of the tube extending into the micropylar haustorium (Figs. 94, 95).

The first division of the zygote is usually transverse, producing a small apical cell, *ca*, and a long tubular basal cell, *cb* (Fig. 103). The main body of the endosperm, excluding the haustoria, is about 16-celled at this stage (Fig. 94). In one case the endosperm proper consisted of about 23 cells, but the zygote had not divided. The basal cell, *cb*, divides transversely into two cells, *m* and *ci*, and the apical cell, *ca*, divides longitudinally, producing two juxtaposed cells, *q*. The wall may not be quite vertical but oblique (Fig. 104). The 4-celled proembryo is T-shaped (i.e. if seen with its apex directed upwards). The two juxtaposed apical cells, *q*, divide transversely into four quadrants which are arranged in two tiers, *l* and *l'*. The proembryo is now 6-celled (Fig. 107). The next stage shows the two cells of the tier *l* to have divided longitudinally into four cells. The two quadrant cells in the tier *l'* have remained undivided. The proembryo at this stage consists of 8 cells (Fig. 108). Had the two cells of the tier *l'* also divided like those of the tier *l*, this could be said to constitute the usual octant stage. The 8-celled stage of the proembryo may not always be derived in a regular fashion. Figs. 110, 111 show that frequently the divisions may be irregular. No regularity of sequence in division of cells can be discerned in later stages. The cells *m* and *ci* seem to produce only a few cells that form a slender suspensor. The other cells give rise to the main body of the embryo which is undifferentiated.

A frequent variation in the mode of division of the zygote is that the wall is longitudinal. The wall, however, does not divide the zygotic tube into two equal cells extending from one end to the other. It meets the tube on one side and cuts off an apical cell which is situated laterally. The other cell, continuous with the tube, corresponds to the basal cell (Fig. 105). The laterally situated apical cell divides longitudinally and the basal cell transversely (Fig. 106). The only difference that this mode of first division makes is that the apical cell and its derivatives come to be situated on one side of the

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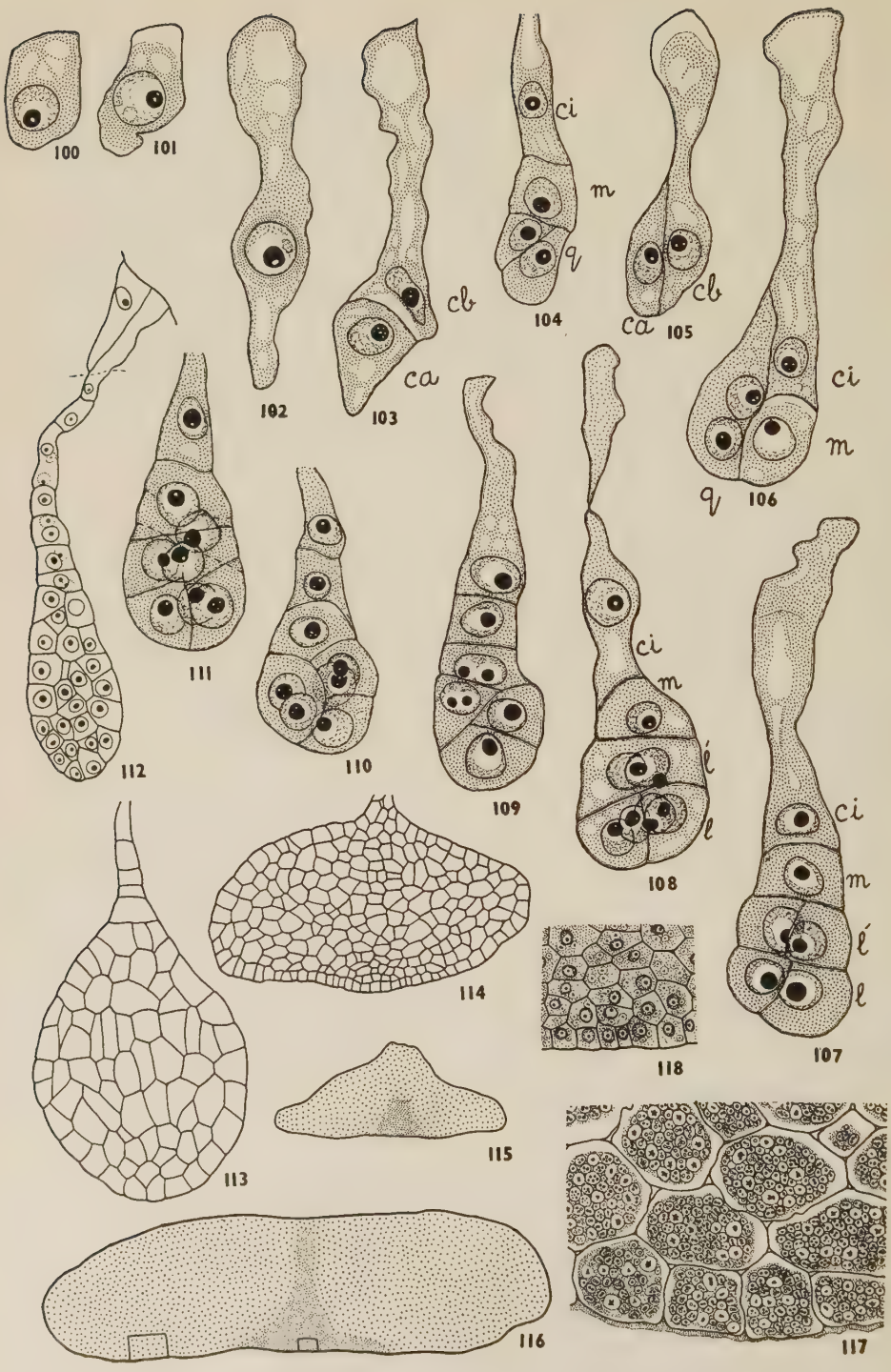
FIGS. 96-99 — *Utricularia flexuosa*. Fig. 96. Upper region of endosperm and part of embryo. A large proportion of placental nutritive tissue has been consumed and many of its nuclei are seen in the micropylar haustorium. The two nuclei of the haustorium are irregular in shape. Note differentiation of plug in upper part of endosperm proper (reconstructed). Fig. 97. Micropylar haustorium at an older stage. The small nuclei in the haustorium belong to the placental nutritive tissue. The larger nuclei have probably been derived from the two original nuclei of the haustorium. Fig. 98. L.S. of an ovule, showing abnormal endosperm. The micropylar haustorium has developed a lobe which is in contact with an adjacent ovule. The chalazal haustorium is 3-nucleate (reconstructed). Fig. 99. Young abnormal endosperm consisting of seven cells arranged in a linear row. The zygote is seen inside the uppermost cell (reconstructed). Figs. 96, 98. × 394. Fig. 97. × 330. Fig. 99. × 738.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FIGS. 100-118.

apex instead of being placed at the tip. Arrangement of cells in embryos developing in this way seems irregular from the very beginning. This mode of development may be regarded as one of the causes that bring about the production of embryos in which the growing point is situated laterally. This peculiar mode of the first division of the zygote also enables one to say definitely that the 4-celled, T-shaped proembryo is derived by longitudinal division of the apical cell and transverse division of the basal cell although actual division was not observed.

In one proembryo, which had reached the 6-celled or the quadrant stage, the disposition of the different walls was such as to indicate that the proembryo was linear at the 4-celled stage (Fig. 109). The two cells produced by transverse division of *ca* had divided, producing four quadrants. This sequence of divisions conforms to that seen in the Solanad type although the disposition of the four quadrant cells here is rather irregular.

A lack of differentiation in the mature embryo makes it difficult to classify it with any degree of certainty. It may, however, provisionally be suggested that the usual course of embryogeny seems to follow essentially the Catalpa variation of the Onagrad type (see Johansen, 1950; Souèges, 1940) and may occasionally exhibit a sequence of early cell divisions which resembles, more or less, the Solanad type.

The young embryo in *Utricularia flexuosa* is at first club-shaped or pear-shaped. Older stages show the embryo to have grown more in transverse plane than in the longitudinal. The embryo, therefore, becomes spherical, then bun-shaped and may eventually be described as disc-shaped (Figs. 112-116). As the embryo changes in shape, the suspensor becomes less conspicuous, disappearing completely in older stages. The main body of the embryo consists of large polygonal cells which are so closely packed with food-grains that their nuclei can no longer be distinguished (Figs. 116, 117). The central region of the apical side consists of cells that are much smaller. They may extend to a longer or shorter distance towards the basal end of the embryo along its axis (Figs. 116, 118). No cotyledons, plumule or radicle could be distinguished in the oldest embryo examined. The entire mature embryo is an undifferentiated disc-shaped body consisting of two kinds of cells (Figs. 116-118).

Young embryos are broader and convex towards the apex or the chalazal end and they taper towards the basal, suspensor end. In one case the apical end was also seen to have become narrow and pointed and looked somewhat like a suspensor (Fig. 119). An exceptional ovule contained two young embryos (Fig. 120). It may be that a synergid became fertilized and developed into an embryo or the ovule might have contained two embryo sacs the eggs of which became fertilized and developed further. One ovule possessed two embryos that were partly joined in the manner of

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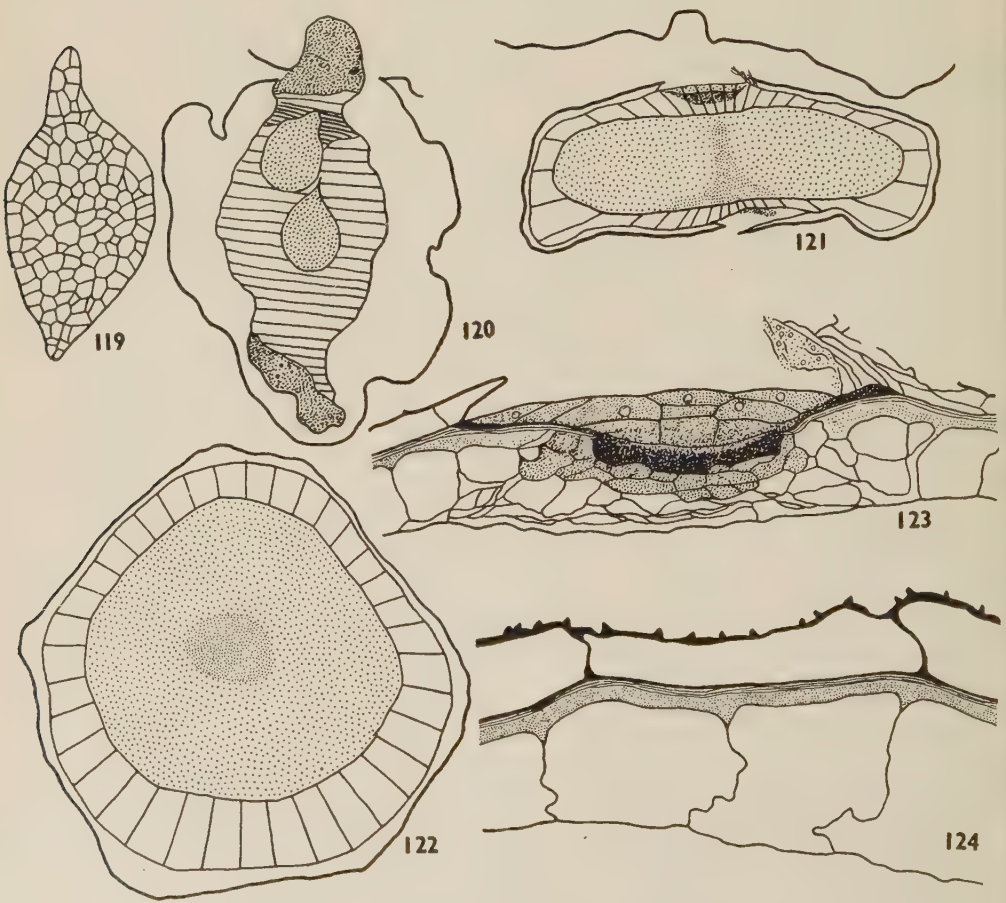
FIGS. 100-118 — *Utricularia flexuosa*. Figs. 100-102. Zygote showing stages in development of zygotic tube (Fig. 102 is a reconstruction). Fig. 103. 2-celled proembryo formed by transverse division of zygote. Fig. 104. 4-celled proembryo. Fig. 105. 2-celled proembryo formed by longitudinal division of zygote. Fig. 106. 4-celled proembryo derived from the condition shown in Fig. 105 (reconstructed). Figs. 107, 108. 6- and 8-celled stages of proembryo (reconstructed). Fig. 109. 6-celled proembryo showing disposition of walls which indicates that the proembryo was linear at 4-celled stage. Figs. 110, 111. 8-celled proembryos derived from irregular divisions (reconstructed). Fig. 112. L.S. of older embryo. The persistent synergid is seen at the base of suspensor. The broken line indicates the boundary between cellular endosperm and micropylar haustorium (reconstructed). Fig. 113. The embryo is becoming spherical. Fig. 114. Older embryo showing further change in shape. The cells near the apex are smaller. Fig. 115. Outline of embryo which has become bun-shaped. Fig. 116. Outline of an old embryo which is now disc-shaped. Note the two regions shown with large and small dots. The plane of section of all the embryos is longitudinal. Fig. 117. Cells of embryo from larger rectangle in Fig. 116. Fig. 118. Cells of embryo from smaller rectangle in Fig. 116, drawn at same magnification as those in Fig. 117. Figs. 100-102, 104-111. × 762. Fig. 103. × 728. Figs. 112, 113, 117, 118. × 406. Fig. 114 × 170. Figs. 115, 116. × 70.

Siamese twins. This may be explained either as resulting from a cleavage of a single embryo or from fusion of two embryos lying close together.

The growth of the zygote into a long tubular structure is a common feature in the Tubiflorae. However, the mode of development of the zygotic tube as described here has been reported earlier only in *U. coerulea* by Kausik (1938). The sequence of early cell divisions in the embryo is known in very few species of the family Lentibulariaceae. In *U. coerulea* the 4-celled proembryo is linear. In *U. vulgaris americana* the 4-celled proembryo, as figured by Wylie and Yocom (1923), is T-shaped. A consideration of the earlier work, as cited by Johansen (1950), and its recent confirmation by Crété (1951), leads to the conclusion that the 4-celled proembryo in *U. vulgaris* may be either T-shaped or linear. This is similar to the condition in *U. flexuosa*. The mature embryo in all the species of



FIGS. 119-124 — *Utricularia flexuosa*. Fig. 119. Abnormal embryo with both ends pointed. Fig. 120. Ovule with two embryos (reconstructed). Fig. 121. Seed in l.s. The line above the seed marks limit of placenta. The pit in placenta represents micropylar haustorium. A corresponding depression is seen in seed (reconstructed). Fig. 122. Seed in t.s. (reconstructed). Fig. 123. Part of seed seen in Fig. 121 magnified to show plug and torn funicle. Fig. 124. Part of seed shown in Fig. 121 illustrating a portion of seed coat (upper cells with projections on outer surface) and remains of endosperm (lower layer of cells). Figs. 119, 123. × 208. Fig. 120. × 90. Figs. 121, 122. × 48. Fig. 124. × 486.

Utricularia studied so far usually shows no more differentiation than that of the epidermis and the growing point. The latter may be situated apically or shifted laterally. In some species regarded as viviparous, e.g. *U. reniformis* and *U. nelumbifolia* (Merl, 1915), the embryo bears two or more primordia of what have been termed the primary leaves. Cotyledons are known to differentiate in *Genlisea* and *Pinguicula*, but the latter alone has a root primordium.

The embryogeny in the various families of the Tubiflorae is variable. Of the five types of embryogeny known to occur in the order, the Onagrad type seems to be the commonest (see Schnarf, 1931; Crété, 1942). In addition to the Lentibulariaceae, reduced embryos occur in the order in *Cuscuta*, Lennoaceae and Orobanchaceae.

Seed

When the young embryo is pear-shaped or spherical, the cellular endosperm becomes differentiated into three parts (Fig. 95). The contents of some cells at the chalazal end become dense. The cells in the middle part, in which the embryo is embedded, are large and vacuolate. In the upper part the cells become differentiated into two kinds. The cells adjacent to the micropylar haustorium are large in size while those situated further away from the haustorium are smaller and have dense contents (Figs. 95, 96). In the oldest seed examined the chalazal haustorium and the endosperm cells at this end are seen to have become crushed (Fig. 121). The disc-shaped embryo is surrounded by a single layer of endosperm cells (Figs. 121, 124). The micropylar part of the endosperm proper, which had become differentiated into two regions, now forms a structure described as a plug (Figs. 121, 123). It cuts off communication between the micropylar haustorium and the endosperm proper. The cells of the integument, including the endothelium, are gradually crushed. All the cells of the integument eventually disappear except the epidermis which forms the seed coat. The epidermal cells become thick-walled and develop numerous short, pointed, spine-like projections on the outer surface (Fig. 124). The seed is flat and polygonal or almost circular (Figs. 121, 122). When it separates from the placenta, the cells of the funicle are torn and the micropylar haustorium, which is still swollen, becomes ruptured. The haustorium continues to be represented in the placenta by a cavity and in the seed by a shallow depression, lined at the bottom by the plug (Figs. 121, 123).

The structure of the seed in *Utricularia flexuosa* is essentially like that of the seed in other species of the genus. The formation of the plug from the cells of the endosperm proper was reported by Merz in 1897 and has also been described in *U. vulgaris americana* (Wylie & Yocom, 1923).

Discussion

The family Lentibulariaceae had been at one time connected with the Primulaceae mainly on the basis of the free central placenta (see Kamienski, 1897; Wettstein, 1935). At present there seems, however, to be no doubt that its nearest relatives are to be found among the Scrophulariaceae. In floral structure it resembles the more advanced Scrophulariaceae and represents a high degree of zygomorphy. The unilocular ovary with its free central placenta presents no difficulty in its derivation from the condition in Scrophulariaceae (see Wernham, 1912). Both Scrophulariaceae and Lentibulariaceae include members in which the apex of the embryo sac grows out of the ovule. This common feature, however, need not necessarily be regarded as indication of relationship. Well-developed endosperm haustoria, which are a characteristic feature of the Scrophulariaceae, are also present in most members of the Lentibulariaceae. The development of endosperm in those members of the Lentibulariaceae in which it is known with certainty conforms to the Scutellaria type and not to the types usually seen in the Scrophulariaceae. Differences of such a nature in endosperm can sometimes occur within the limits of a single family. The undifferentiated embryo in the Lenti-

bulariaceae also presents no difficulty in tracing relationship with the Scrophulariaceae since it has obviously been derived from typical dicotyledonous condition by reduction. The 4-celled proembryo in some Lentibulariaceae is linear, but the T-shaped condition, which is common in Scrophulariaceae, is also known to occur.

From the morphological point of view the genus *Pinguicula* is obviously the least specialized member of the family. *Genlisea* comes next and is followed by *Polypompholyx* and *Utricularia*. *Biovularia*, with only two ovules, also represents a derived condition. A comparative study of embryological characters leads to a sequence which agrees with the series based on comparative morphology although it is not possible to determine the position of *Biovularia* which has not so far been investigated embryologically.

Pinguicula is not known to possess a nutritive tissue, either in the ovule or in the placenta. *Genlisea*, next in the series, has chalazal and micropylar groups of nutritive cells within the ovule. In *Polypompholyx* the nutritive tissue corresponding to the micropylar group of cells in the preceding genus, is shifted to the funicle. In *Utricularia*, representing the culmination of the series, this tissue develops in the placenta. The embryo sac remains completely within the ovule in *Pinguicula* and *Genlisea*. In *Polypompholyx* the apex of the embryo sac grows beyond the ovule. In *Utricularia* it enters the nutritive tissue in the placenta. It is not possible to construct a series on the basis of development of the endosperm because the sequence of early cell divisions is known with certainty only in a few members of the family. In all these it conforms to the Scutellaria type. A different mode of development, assumed to be seen in *Utricularia vulgaris americana*, seems to be rather doubtful. The behaviour of the micropylar endosperm haustorium is associated with the development of the nutritive tissue and the behaviour of the embryo sac. It, therefore, exhibits a similar gradation. In *Pinguicula* the micropylar haustorium is absent. In *Genlisea* this haustorium is present, but remains within the ovule. In *Polypompholyx* it enters the nutritive tissue at the base of the funicle. In *Utricularia* the micropylar haustorium is situated in the placental nutritive tissue. The embryo exhibits a similar sequence. The two least specialized genera show the nearest approach to the typical dicotyledonous embryo while *Utricularia*, with reduced and undifferentiated embryo, represents the most highly evolved stage in the series.

Summary

The general course of embryology in *Utricularia flexuosa* agrees with that in related plants, but it exhibits a remarkable degree of variation in almost every aspect of its development.

During the development of flower, the perianth and androecium precede the gynaeceum which is initiated last of all.

The ovules are anatropous, tenuinucellate and unitegmic. Hemianatropous condition occurs occasionally. The chalazal nutritive tissue is present. The nucellus degenerates early and the developing embryo sac becomes naked. The ovule has no micropyle in the strict sense. The integumentary tapetum is well developed and is in direct contact with the embryo sac.

There is usually a single hypodermal archesporial initial cell, but occasionally there may be two. The archesporial cell functions directly as the megaspore mother cell. In one case three mother cells were seen in the same ovule. The megaspore tetrad is usually linear, but sometimes it may be T-shaped, or intermediate between linear and T-shaped, or isobilateral; occasionally the two middle megaspores are situated side by side, resulting in a crosswise arrangement of the megaspores. Usually the chalazal megaspore alone is functional. Old tetrads often show more than one healthy megaspores. In exceptional cases all the four megaspores seem to be potentially functional. Occasionally the megaspore nucleus exhibits a budding appearance or the megaspore may contain a small supernumerary nucleus.

The development of the embryo sac conforms to the Polygonum type. The naked apex of the mature embryo sac penetrates into the placental nutritive

tissue. The embryo sac is in direct communication with the ovarian cavity through the passage between the integument and the placenta. The pollen tube approaches the embryo sac through this passage.

In more than a dozen embryo sacs there were three polar nuclei and only one synergid. In one sac there were four polar nuclei and no synergid at all. Occasionally a synergid resembles the egg in appearance. An 11-nucleate embryo sac with five nuclei in the position of the polars has also been observed. An embryo sac at the 4-nucleate stage of development exhibited reversion of polarity. Twin embryo sacs are frequent and occasionally even three or four embryo sacs occur in the same ovule.

The anther has four, occasionally five, pollen chambers. The hypodermal archesporium consists of a single layer of cells. The wall of the pollen chamber consists of the epidermis, the endothecium with well-developed fibrillar thickenings, a middle layer and the tapetum. The microspore tetrads are usually tetrahedral and occasionally decussate or rhomboid. The pollen grains are of two types. In the commoner type the exine has 14-19 broad longitudinal strips, alternating with narrow furrows. In the second type the surface of the grain is divisible into four tetrahedrally disposed areas. Strips of exine starting from the centre of one area pass into the three adjacent areas and approach their centres. The mature pollen is 3-nucleate. Sometimes the pollen grains germinate in the anther.

Giant pollen grains occur occasionally. 4-celled compound pollen grains produced by non-separation of microspores of a tetrad and development of common exine occur frequently. Very often the microspores contain two equally large nuclei separated by a thin wall dividing the microspores into two equal cells, or the wall may be absent. Pollen grains containing supernumerary nuclei or what may be described as double gametophytes have been observed. One pollen grain contained more than half a dozen nuclei of different sizes. In one case there were one large and three small nuclei. This may be a case of polyspermy.

After travelling some distance in the wall of the style, the pollen tubes enter the stylar canal, approach its base and then pass over to the placenta. Some tubes creep along the ovary wall for some distance and then cross over to the placenta. One synergid is destroyed by the pollen tube while the other may persist for a long time. Double fertilization has been observed. Structures comparable to X-bodies are common.

The sequence of early cell divisions in endosperm development conforms to the Scutellaria type and produces the 8-celled endosperm consisting of four tiers of two cells each. The wall separating the cells of the terminal pairs is incomplete. The uppermost tier develops into the micropylar haustorium which is 2-nucleate when young. The lowest tier develops into the 2-nucleate chalazal haustorium. The four middle cells give rise to the endosperm proper. The contents of some of the cells of the endosperm proper, situated adjacent to the micropylar haustorium, often acquire resemblance to the contents of the haustorium. The walls of some of them break down and the contents become incorporated into the haustorium which becomes multinucleate. Some of the cells which become so modified, remain intact and the haustorium appears to be multicellular. The aggressive micropylar haustorium penetrates deep into the placental nutritive tissue whose cells break down and their nuclei enter the haustorium. The number of placental nuclei seen in the haustorium may exceed 150. The original nuclei of the haustorium in older stages break up into several separate nuclei. The haustorium had, in one case, developed a lateral lobe which was protruding out and had come in contact with an adjacent ovule. The chalazal haustorium is also very aggressive. Occasionally it contains three nuclei.

In one ovule the young endosperm consisted of 7 cells arranged in a row.

A small protuberance appears at the apex of the zygote and grows into a long tube. The nucleus migrates into this zygotic tube which penetrates into the cellular endosperm. The zygote usually divides transversely. The 4-celled pro-

embryo is T-shaped. The two apical cells divide transversely to produce the four quadrants placed in two tiers. The two cells of the apical tier divide longitudinally into four. Further divisions are irregular. The mature embryo is undifferentiated.

The zygote often divides by a longitudinal wall. The 4-celled proembryo is occasionally linear. A young abnormal embryo exhibited a suspensor-like protrusion at the apex in addition to the usual suspensor. Polyembryony occurs occasionally.

During seed development the endosperm is reduced mainly to a layer of cells surrounding the embryo and the plug that cuts off communication with the micropylar haustorium. The integument is reduced to a single layer of cells which forms the seed coat.

The relative degree of specialization in the various genera as revealed from their comparative embryology is in general agreement with that based upon a comparative study of the morphological characters.

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STRUCTURE AND DEVELOPMENT OF SEEDS IN EUPHORBIACEAE: *RICINUS COMMUNIS* L.

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The family Euphorbiaceae, though well represented in India, has not received as much attention as it deserves. Most published accounts[1] deal with the development and organization of the female gametophyte, except that of Johri and Kapil (1953). Investigations on the development and structure of the seed have been practically neglected.

Netolitzky (1926) has reviewed the literature on the family, but so far no attempt has been made to classify the various genera on an anatomical basis. It is well known that the seeds of the genera of some sub-tribes possess a caruncle while those of others do not (Pax & Hoffman, 1931). Similarly, different types of vascularization have been reported in the seeds of this family (Netolitzky, 1926; Landes, 1946).

Since the author expects that seed structure also may be helpful in the classification of different genera, the present investigation has been taken up, and this paper is the first of the series.

Material and Methods

The material for the present study was collected from the Botanical Garden, B.R. College, Agra.

Flowers, ovules and seeds at different stages of development were fixed in formalin-acetic-alcohol. They were dehydrated and run through the alcohol-xylol as well as the tertiary-butyl alcohol series. Serial sections (8 to 16 μ in thickness) were stained with Heidenhain's iron-alum-haematoxylin, Delafield's haematoxylin and safranin-fast green combinations. Macerations of the sclerenchymatous layer of the seed coat were done with Jeffery's solution.

Observations

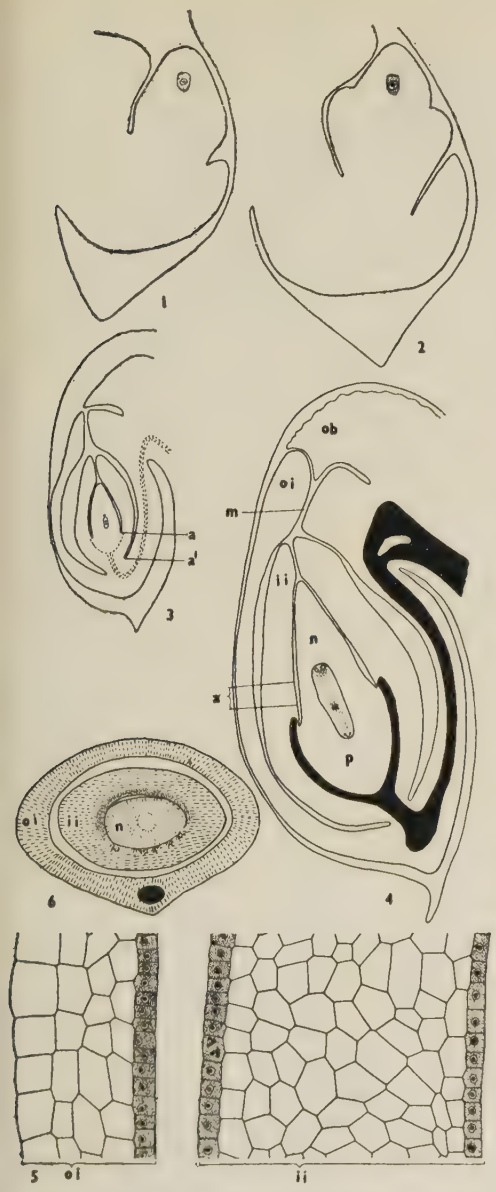
Ovule — A single ovule arises as a small outgrowth on the axile placenta in each loculus of the tricarpellary, syncarpous, trilocular ovary. It finally becomes pendulous and anatropous with the micropyle facing upwards. The outer integument appears first followed by the inner (Figs. 1, 2). As the growth of the ovule proceeds, the developing embryo sac becomes deep-seated due to divisions of the parietal and epidermal cells of the nucellus. The two integuments, which grow faster than the nucellus, fully encase the latter at the binucleate embryo sac stage (Fig. 3). The micropyle is formed by both the integuments but the exostome and endostome do not lie in one line (Fig. 4) except in the earlier stages as shown in Fig. 3.

Fig. 2 shows that the inner integument possesses a broad base and its inner margin arises at a much higher level than the outer.

Active proliferation at the chalazal end of the nucellus is noticeable at the binucleate embryo sac stage or even earlier, which continues even during the development of the seed. Due to this proliferation the distance between the basal limit of the inner margin of the inner integument and the chalaza increases considerably (Fig. 4).

Although the nucellus becomes massive at the mature embryo sac stage, its apex never extends beyond the base of the endostome (Fig. 4) as is also the case in *Putranjiva roxburghii* and *Trewia nudiflora* (Banerji & Dutt, 1944).

1. Kajale & Rao, 1943; Banerji & Dutt, 1944.

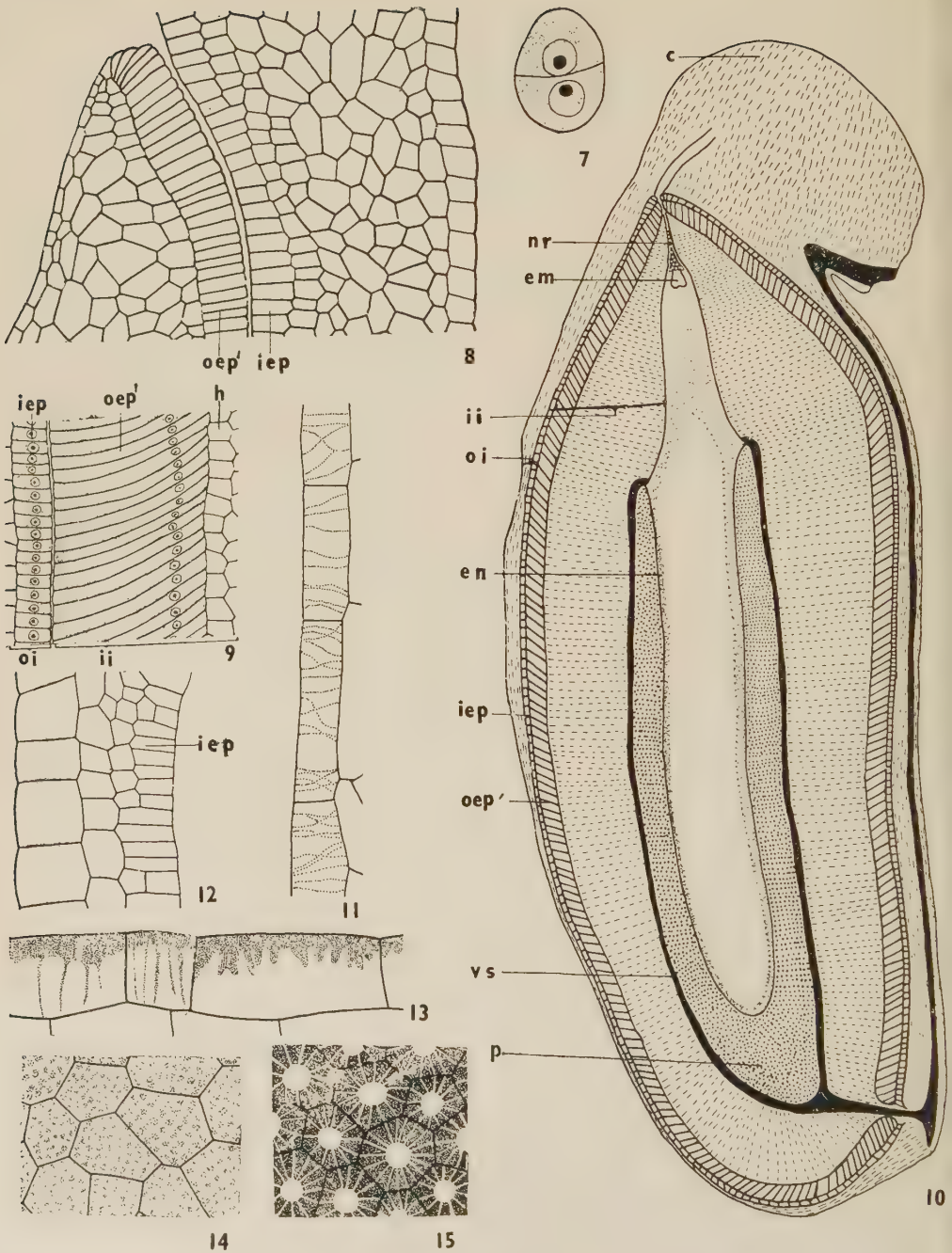


FIGS. 1-6. (*ii*, inner integument; *m*, micropyle; *n*, nucellus; *ob*, obturator; *oi*, outer integument; *p*, proliferating area.) Fig. 1. L.S. ovule with megaspore mother cell, well-marked outer integument and initiation of inner integument. × 162. Fig. 2. Same, older stage. Vascular supply not shown. × 162. Fig. 3. L.S. ovule with binucleate embryo sac. Mark the basal limit *a*-*a'* of the inner integument and the upper limit of the vascular supply. × 40. Fig. 4. L.S. ovule at the mature embryo sac stage. Compared with Fig. 3 the basal limit of both margins of inner integument shows an increase in distance between them. Note portion of obturator projecting into the micropyle. × 50. Fig. 5. Enlarged view from Fig. 4 of portion *a*. × 422. Fig. 6. Slightly oblique section with the vascular bundles in a ring. Note separation of nucellus from inner integument from side opposite to funiculus, and presence of diffused vascular supply on the side as contrasted with clearly outlined vascular bundles on other. × 50. *N.B.* — Vascular supply is not shown in Figs. 1 and 2.

The outer epidermis of the outer integument is formed of larger cells with weakly staining cytoplasm. The inner epidermis of the outer integument and both the epidermal layers of the inner integument are formed of more or less isodiametric cells with densely staining cytoplasm and mostly centrally placed nuceli (Fig. 5). The cells lying between the epidermal layers of both integuments are polygonal, vacuolate and parenchymatous. The obturator, which is formed as an outgrowth of the placenta, is fairly well developed and a small portion of it projects into the micropyle (Fig. 4). The epidermal cells of the obturator, which are at first nearly isodiametric, become elongated and finger-shaped with granular contents as described in *Euphorbia* by Kajale and Rao (1943). Rest of the cells form a more or less compact mass.

The vascular strand of the ovule takes a sharp turn from the placenta into the funiculus; it passes down through the raphe and enters the chalaza (Figs. 3, 4). Here it is divided into a number of branches that ramify and arrange themselves into a ring (Fig. 6). This ring of vascular bundles ascends up and ultimately ends near the inner margin of the inner integument at the place where it separates from the nucellus. There is no vascular supply entering the free part of the nucellus nor were any vascular elements seen in the proliferating area which immediately lies within the ring of vascular strands (Fig. 4).

ENDOSPERM AND EMBRYO — Marked anatomical changes occur in the ovule in post-fertilization stages. The first of these changes is the development of a free nuclear endosperm. Its nuclei are distributed along the periphery of the embryo sac. The development of embryo starts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FIGS. 7-15.

soon after the endosperm nucleus has undergone three to four divisions. The first division of the zygote is transverse (Fig. 7). By repeated divisions the embryo soon acquires a spherical shape and shows a differentiation into the cotyledons, the plumule and the radicle much before the endosperm has become completely cellular.

Changes in the Nucellus and the Chalaza — Simultaneously with the development of the embryo and the endosperm, changes occur in nucellus, chalaza and the two integuments. The first of these changes concerns the cells of the free part of the nucellus, which enlarge in size and become vacuolate, while the cells on the chalazal side divide actively. As the development of the seed proceeds, most of the free part of the nucellus gets crushed and absorbed by the developing embryo and endosperm leaving only a conical strip to which the embryo is attached (Fig. 10).

The active proliferation of chalazal area is the factor responsible for most of the enlargement of the seed. This part is surrounded by the ring of vascular strands, which are the ramifications of the main chalazal supply. Due to its activity the distance between the bases of the outer and inner margins of the inner integument increases considerably. Simultaneously, the mesophyll cells of the inner integument and the chalazal cells, lying in continuity with the former, grow downwards to keep pace with the growth of the main proliferating portion.

The cells of the proliferating area are more compact and smaller in size than the mesophyll cells of the inner integument and the chalazal cells lying in continuation with it. The mesophyll cells of the inner integument and the chalazal cells lying in continuation with it are similar in shape and structure and thus there is no clear boundary between them.

With the further development of the endosperm, followed by wall formation, which starts from the periphery, the mass of proliferating cells is pushed laterally (Fig. 10). As the seed approaches maturity, these cells are mostly crushed and ultimately form a layer which lines a major part of the seed coat.

Changes in the Inner Integument — The first visible change in the inner integument, besides the increase in its size, is the radial elongation of the outer epidermal cells in the micropylar region so as to give a palisade-like appearance (Fig. 8). In later stages this elongation also extends to the lower part. During the course of development these palisade-like cells become obliquely bent (Fig. 9) and later their walls become sclerified and show simple pit pairs. In the mature seed this layer forms the characteristic sclerenchymatous sheath, also reported for other members of the family (Netolitzky, 1926; Landes, 1946; Johri & Kapil, 1953). The cells of this layer situated at the micropylar end are longer than those at other places. As development proceeds, the micropyle is nearly occluded but the sclerenchymatous layer remains discontinuous on the chalazal side through which the vascular bundle enters the chalaza (Fig. 10). The hypodermis is formed of smaller cells which ultimately get crushed.

←

Figs. 7-15. (*c*, caruncle; *em*, embryo; *en*, endosperm; *h*, hypodermis; *iep*, inner epidermal cells of *oi*; *ii*, inner integument; *nr*, nucellar remains; *oep'*, outer epidermal cells of *ii*; *oi*, outer integument; *p*, proliferated area; *vs*, vascular supply.) Fig. 7. Two-celled proembryo. × 566. Fig. 8. L.S. integuments in the micropylar region showing radially elongated (1) outer epidermal cells of inner integument and (2) inner epidermal cells of outer integument. × 366. Fig. 9. L.S. showing inner epidermal cells of the outer integument, outer epidermal cells of the inner integument and the hypodermis. × 216. Fig. 10. L.S. young seed. × 10. Fig. 11. Inner epidermal cells of inner integument showing thickened strips on their tangential walls. × 566. Fig. 12. L.S. part of outer integument near micropyle showing tangential divisions in its inner epidermal cells. × 566. Fig. 13. L.S. showing outer epidermal cells of outer integument with thickened areas on its outer tangential wall. × 566. Fig. 14. Outer epidermal cells of outer integument in surface view. × 216. Fig. 15. T.S. macrosclereids obtained from sections cut tangentially to the seed. × 566.

The cells of the inner epidermis elongate tangentially and gradually lose their contents. Later on a number of thickened strips develop on their tangential walls (Fig. 11) (cf. Landes, 1946).

The mesophyll cells of the inner integument enlarge in size and slowly lose their contents. During further development of the seed the integumentary cells lying within the sclerenchymatous layer are crushed and form a part of the inner seed coat.

Changes in the Outer Integument — Even before fertilization, the inner epidermal cells divide tangentially near the micropyle (Fig. 12), due to which the outer integument becomes much bulkier in this part. After fertilization, this activity is continued.

After fertilization the outer epidermal cells undergo some tangential elongation. In the later stages of development thickened areas projecting into the lumen develop from the outer tangential wall (Fig. 13; cf. Netolitzky, 1926). The mature seed derives its dark brown colour often stippled with light brown patches due to the contents of the cells of this layer.

The inner epidermal cells become columnar and have a shining appearance. The mesophyll cells elongate tangentially and lose their contents. They are ultimately crushed. On the obturator side air spaces develop which probably help the seed in getting detached from the fruit.

There is a well-developed caruncle formed by the divisions of the micropylar cells of the outer integument (cf. Landes, 1946). The growth of the obturator stops after fertilization and with the growth of the caruncle it is pushed towards the placental side.

Mature Seed — The dorsal surface of the seed is slightly convex while the ventral surface has a projecting ridge in the centre formed by the remains of the raphe and the raphal bundle. Spirally thickened xylem elements are present in this ridge.

As stated above, the outermost coloured layer of the mature seed is derived from the outer epidermis of the outer integument. In surface view its cells are polygonal with no intercellular spaces (Fig. 14). Next come the crushed cells of the mesophyll and then the inner epidermal cells of the outer integument. All these three portions together form the outer seed coat.

The inner seed coat, formed by the inner integument, gets differentiated into two portions. The outer one is derived from the sclerenchymatous layer. In cross-section these cells of sclerenchyma appear polygonal with greatly thickened walls (Fig. 15). The inner one is formed by the remaining layers of the inner integument in continuation with the crushed cells of the chalaza. The vascular supply of the chalaza persists in somewhat crushed condition.

As mentioned before, the layer formed by the crushed proliferated cells persists and lines a major part of the inner seed coat.

The cells of the endosperm are filled with food reserves, particularly oil. It occupies the major volume of the mature seed.

The embryo occupies the full length of the seed and shows a well-marked root cap. Vascular elements are in the form of procambial cells.

Summary

The ovules are bitegmic and anatropous with the micropyle facing upwards. The outer integument arises first. The micropyle is formed by both the integuments but the exostome and endostome do not lie in one line.

The embryo sac becomes deep-seated due to the divisions of the parietal and the epidermal cells of the nucellus.

The vascular supply of the ovule terminates at the basal part of the inner integument and does not extend to the free part of the nucellus.

The first division of the zygote is transverse and the embryo occupies the full length of the seed.

The endosperm is free nuclear but later becomes cellular and occupies the major part of the seed.

An important feature is the proliferation of the cells at the chalazal side. This plays a very important role in the enlargement of the seed.

The inner seed coat, formed by the inner integument, is differentiated into two parts. The outer one, formed by the outer epidermal cells, is sclerenchymatous, while the inner one, formed by the remaining layers and the crushed chalazal cells lying in continuation of the former, is papery.

The outer seed coat is derived from the outer integument. It is formed by (a) the outer epidermal cells, (b) crushed cells of mesophyll, as well as (c) the inner epidermal cells of the outer integument.

The caruncle is formed by the divisions of the micropylar cells of the outer integument.

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CYTOLOGICAL CHANGES ACCOMPANYING ABSCISSION OF PERIANTH SEGMENTS OF *MAGNOLIA GRANDIFLORA*

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In this study, the cellular changes in abscission of perianth segments of *Magnolia grandiflora* L. were observed and recorded and the cells of the abscission zone were compared with adjacent cells in the receptacle and with meristematic cells of the floral bud. This species was particularly adaptable for this work because the processes leading to abscission may extend over a period of 2 or 3 days. Further, the abscission zone is extensive, due to the thickness of the segment, making possible observation of progressive changes during separation.

The attention of previous workers has been directed to three phases of the cytological processes accompanying abscission of fruits and floral parts: (1) the meristematic condition of the abscission zone, including cell division, (2) cell wall changes

that occur during separation, and (3) starch grain distribution in relation to the abscission zone. Retention of meristematic conditions by abscission zones in pedicels has been reported for several species of the Solanaceae (Kendall, 1918), sweet-pea buds (Nightingale & Farnham, 1936), young fruits and flowers of the mango and avocado (Barnell, 1939), and male flowers of *Mercurialis annua* (Yampolsky, 1934). In addition, cell division was observed in similar zones in flowers and young fruits of apples (McCown, 1943). Cell wall changes in the separation layer occur during abscission. Swelling of the wall or portions of it, especially the middle lamella, was found to occur by Nightingale and Farnham (1936), Barnell (1939), and McCown (1943). Dissolution of the middle lamella was observed by Kendall (1918) and Yampolsky (1934).

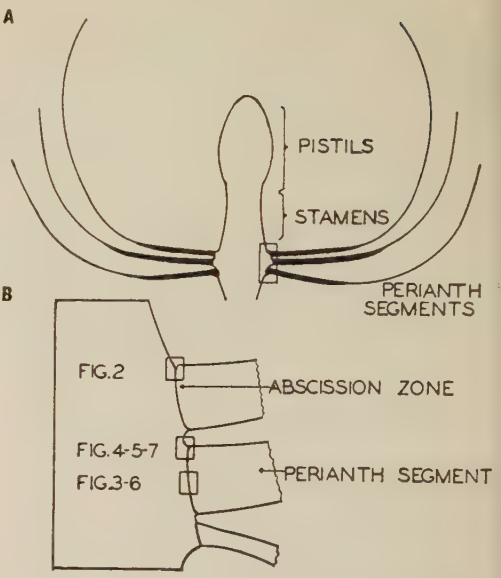


FIG. 1—Radial-longitudinal diagrams through the flower. A. Entire flower. × ½. Box indicates region and extent of Fig. B. B. Region of perianth segment attachment. × 4. Boxes indicate regions of figures as labeled.

Materials and Methods

Flowers from cultivated *Magnolia grandiflora* trees in the Los Angeles area were used. Sectioned material, both fresh and embedded in paraffin, was examined. Material for embedding was fixed in Nawashin's fluid, dehydrated by the n-butyl alcohol method, and embedded in hard paraffin. Sections were cut at 10 μ and stained with Conant's quadruple stain, resulting in certain constant colour patterns.

Green — The cell plate, subsequent to contact with the old wall, and the walls of the meristem stain deep green; walls of older cells are light green. Contents of secretory cells, some grains, and crystallized appearing cytoplasm stain green or grey-green.

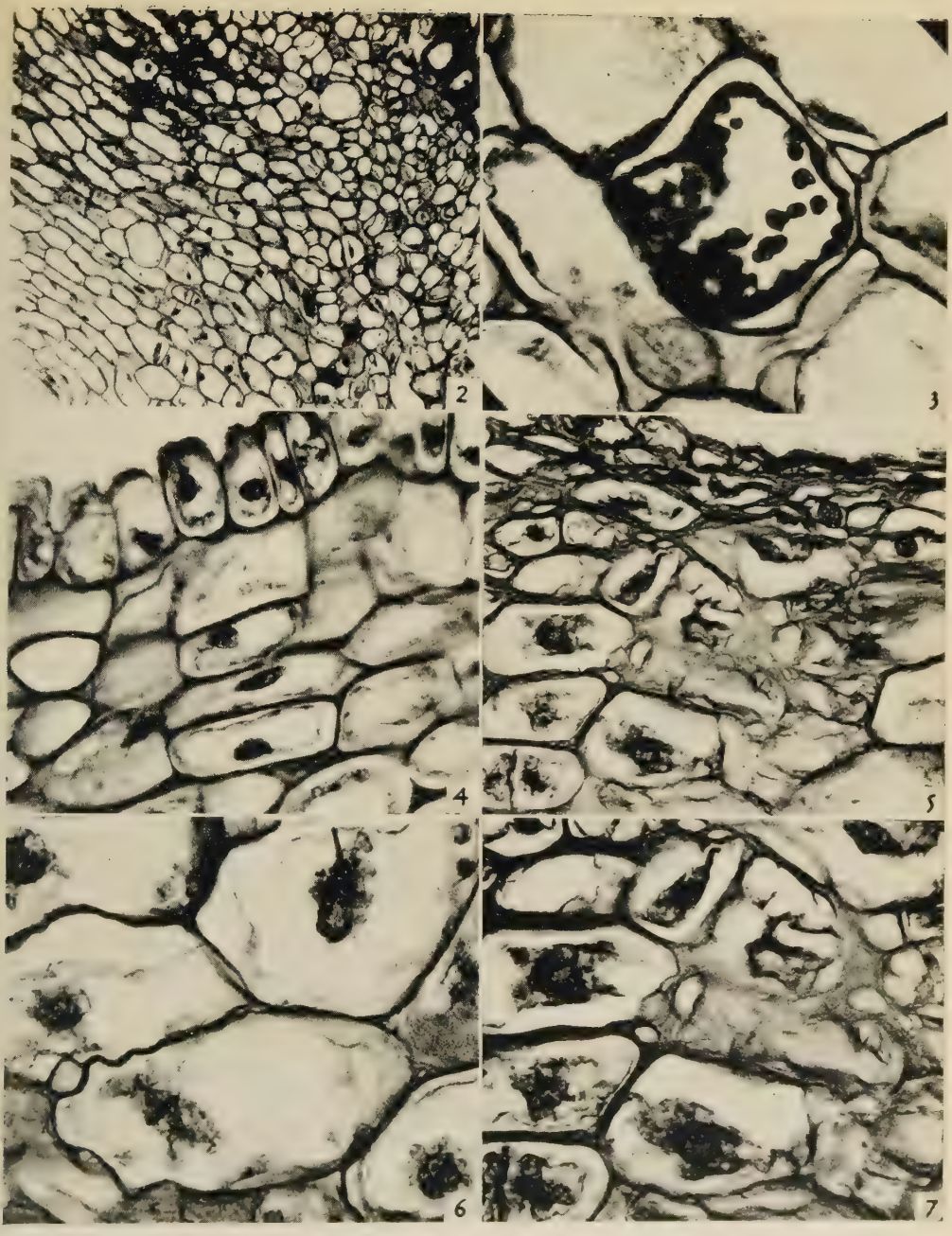
Magenta — The granular portion of cytoplasm, nuclear network, phragmoplasts, and the cell plates prior to contact with the cell wall stain magenta, a combination of crystal violet and safranin.

Red — The walls of the tracheary elements and sclereids stain a purple-hued red. Walls of cells forming the cork of the periderm and material laid down on injured or exposed surfaces stain with a darker cast. Nucleoli stain bright red. In the cytoplasm of some parenchyma cells of the flower, material either in globular form or as a solid mass stains bright red (Fig. 3). Granules that appear in cells, usually in large numbers, on or near exposed surfaces due to injury or abscission, stain red (Fig. 8). In flaccid perianth segments, protoplasm stains bright red.

Lavender — Starch grains stain lavender.

Orange — The cuticle, cell wall thickenings in the hypodermis, surfaces of intercellular spaces, especially in the perianth segments, and walls of sieve elements stain various intensities of orange.

Standard microchemical tests were used on fresh material to identify various substances that were stained as described above. It has been shown by Scott (1950) that waxy substances generally identified as suberin or cutin can best be detected by the IKI-H_2SO_4 test, as described in her paper. Further, Sudan III stains these materials as well as various other oils and waxes. In *Magnolia* the cuticle, thickenings of the hypodermal walls, linings of intercellular spaces, walls of corky tissue, and walls of cells exposed



FIGS. 2-7 — Radial-longitudinal sections through flower receptacle in the region of perianth segment attachment. Fig. 2. Abscission zone of upper segment, showing groove (upper left-corner) and row of dividing cells forming abscission layer. × 85. Fig. 3. Cell containing oil globules × 520. Fig. 4. Normal section of epidermis and hypodermis of perianth segment. × 350. Fig. 5. Epidermis and hypodermis in separation layer showing splitting of cell walls occurring before abscission. × 350. Fig. 6. Abscission layer showing mitotic figure, wall splitting (especially in lower right hand corner), and dextrin-like grains in parenchyma cells (clearly shown in cells in upper and lower right corners). × 520. Fig. 7. Portion of Fig. 5 showing greater detail of cell wall splitting × 520.

to the atmosphere by injury or abscission gave a positive test for suberin or cutin with IKI-H_2SO_4 tests and Sudan III. The phloroglucinol and hydrochloric acid test for lignin showed lignin to be present only in the walls of tracheary elements and sclereids in the region studied.

Cutin, suberin, and lignin were differentially stained by Conant's quadruple stain. The cuticle, cell wall thickenings of the hypodermal cells, and surfaces of intercellular spaces stain orange. Cork and walls of cells exposed to the atmosphere due to injury or abscission stain dark red, while walls of tracheary elements and sclereids stain a purple-hued red. Although other substances may stain red or orange with Conant's quadruple stain, this stain when used in conjunction with the standard microchemical tests gives a differential test for cutin, suberin, and lignin; cutin stains orange, suberin a dark red and lignin a purple-hued red.

Protoplasm stains two distinct colours. The protoplasm of normal living cells stains magenta and has a fibril network in the cytoplasm. Cells of flaccid perianth segments and those near exposed surfaces stain bright red and the fibril network is broken. These two changes in the protoplasm generally occur under adverse conditions and apparently indicate the death of the cell.

Globules present in some parenchyma cells in the flower, which stain bright red with Conant's quadruple stain, also stain red with Sudan III. These globules are presumed to be oil.

To study the distribution of starch and similar storage materials, fresh sections flooded with IKI solution were used in addition to the fixed material. Grains, except in the abscission zone during the abscising process, stain dark purple or blue with IKI and lavender with Conant's quadruple stain. In the abscission zone just prior to and during abscission grains are deposited that are morphologically similar to the starch grains but stain brown or red-brown with IKI and grey-green or green with Conant's quadruple stain.

In publications on starch (Hanes, 1937; Lampitt *et al.*, 1941) evidence is cited showing the necessity of certain minimal chain lengths for formation of certain colour complexes. Chain lengths longer than 12 units are necessary for the blue and blue-violet colours, while dextrins containing 6-12 units usually produce red or red-brown colorations. Therefore, the terms starch grain will be used for the grains staining blue or blue-violet with IKI and dextrin-like grain for the grains staining red-brown with IKI.

In this paper the observations are based on paraffin sections stained with Conant's quadruple stain. The microchemical tests described above were used to confirm interpretations of certain colour patterns.

General and Histological Observations

The perianth segments of *Magnolia grandiflora* normally abscise 2-5 days after the flower has opened, this variation depending on temperature and humidity. Generally a decrease in humidity or an increase in temperature shortens the period between anthesis and abscission. Anthesis occurs between sunrise and noon of the same day. During this day or the next, the stamens shed their pollen and abscise shortly thereafter. The perianth segments are generally white at this time, turning brown after the pollen is shed. This colour change is progressive and usually completed by the day prior to perianth abscission. During the 24 hr. period prior to abscission, the segments rapidly lose turgidity, parts of them becoming dry, especially during hot weather. Normally abscission occurs during the late afternoon or night. During periods of hot weather and low humidity, perianth segments become dry before the normal abscission period and tend to be retained. These segments abscise, however, if slight pressure is applied.

In the region of the perianth segments, the receptacle is divided into pith, vascular cylinder and cortex. The xylem elements in this region are mainly spiral tracheids up to 0·5 mm. in length. The phloem consists of sieve elements that may form short tubes 2-3 cells long. Companion cells and fibres were not observed. The cortex contains a large number of small vascular bundles extending from the vascular cylinder to the various parts of

the flower. A sheath, 2-3 cells thick, surrounds each vascular bundle. These cells are considerably elongated in the direction of the bundles and are at all times densely filled with starch grains. Secretory cells and numerous stone cells are present. The outer 2 or 3 layers of the cortex have heavily thickened walls and form a hypodermis. The perianth segments consist of a large mass of parenchyma with relatively infrequent groups of stone cells, individual secretory cells, a 2-3-layered hypodermis with cells flattened parallel to the surface of the segment (Fig. 4), and a large number of small frequently branching veins. Intercellular spaces are fewer and smaller in the segments than in the cortex, the differences occurring gradually.

A groove, about 0·5 mm. deep, has been observed between the adaxial surface of the upper perianth segments and the receptacle (Fig. 2), but not in the lower segments. Abscission of the upper segments starts below this groove.

The abscission zone is histologically identical with the cortex and appears to be an integral part of it. However, just prior to and during abscission it acts as a separate physiological unit. This zone consists of 4-5 layers of cells and separation usually occurs between it and the perianth segment. Fig. 1 shows the abscission zone, indicating the areas photographed.

Cytological Observations in the Region of Abscission

The cytoplasm of the mature parenchyma cells surrounds a vacuole and consists of a fibril-like network forming a thin peripheral layer in which the nucleus and starch grains are embedded. The nucleus, having 1-3 nucleoli each surrounded by a hyaline area, contains a coarse network which extends throughout.

The pattern of starch distribution changes continually throughout the life of the flower. Furthermore, there is a continual decrease of starch in all regions, from the time the flower opens to the time of perianth segment abscission, although not at the same rate in each. In buds that are in the process of opening, a heavy deposit of starch is present in the parenchyma of the perianth segments and bundle sheaths, sections stained with IKI appearing solid black. The quantity of starch changes abruptly between the perianth segment and abscission zone, being absent, or nearly so, in the abscission zone and gradually increasing in the cortex. In the mature flower, just prior to abscission, starch is absent in the segments. A light deposit is present in the cortex, with some dextrin-like grains, while the bundle sheaths continue to have a relatively heavy deposit. A deposit of dextrin-like grains, morphologically identical with starch grains, occurs at this time in the abscission zone (Fig. 6). Examination of flowers taken 1-4 days after abscission shows an absence or near absence of starch grains and dextrin-like grains.

In the mature flower there are parenchyma cells filled with oil globules measuring up to 5 μ in diameter (Fig. 3). These globules are of fairly uniform size in any one cell, but, in some cells, grade down to the limit of visibility and form a large mass. The nucleus appears normal in structure, colour and size. If one of these cells is in the plane of cell division during abscission, it divides normally. Both starch grains and oil globules are sometimes intermixed within the same cell, indicating that these globules are deposited in, or are part of, the cytoplasm. Cells of this type vary with the physiological age of the flower and are not generally distributed throughout it, being concentrated in the perianth segments and the cortex adjacent to them. They are absent in the pith. Their centre of concentration appears to be in the abscission zone between the perianth segments and the cortex.

The walls of the mature parenchyma cells are 1·0-1·5 μ thick, and are similar. The intercellular spaces are lined with cutin and many contain free globules. There is a deposit of suberin in the cell walls of older tissues, being indicated by a red cast to the walls of the parenchyma cells of flaccid perianth segments and the pith of the flowers whose segments are abscising. Walls of hypodermal cells vary in thickness from 1 to 4 μ (Fig. 4). Those

perpendicular to the surface, or nearly so, resemble the walls of the underlying parenchyma cells in colour, structure and thickness. The walls parallel to the surface are thickest at the point of contact of two adjacent cells. The material added to the cell wall is cutin, nearly or entirely filling the intercellular spaces. Except for the outside wall, the walls of the epidermis are of the same thickness and have the same characteristics as those of the hypodermis. The cuticle is 3-6 μ thick and very irregular on its outer surface(Fig. 4).

Cytological Changes Accompanying Abscission

The earliest cytological sign of abscission is the localized splitting of the cell walls of the hypodermis in the abscission zone (Figs. 5, 7). This may be apparent as early as the period of stamen abscission. The region involved varies in width and may encompass several layers of cells; however, in the final stages of splitting it appears to be confined to a narrow band, thus setting it off sharply from the adjacent areas. Globules of cutin are present in the openings created in the cell walls by splitting.

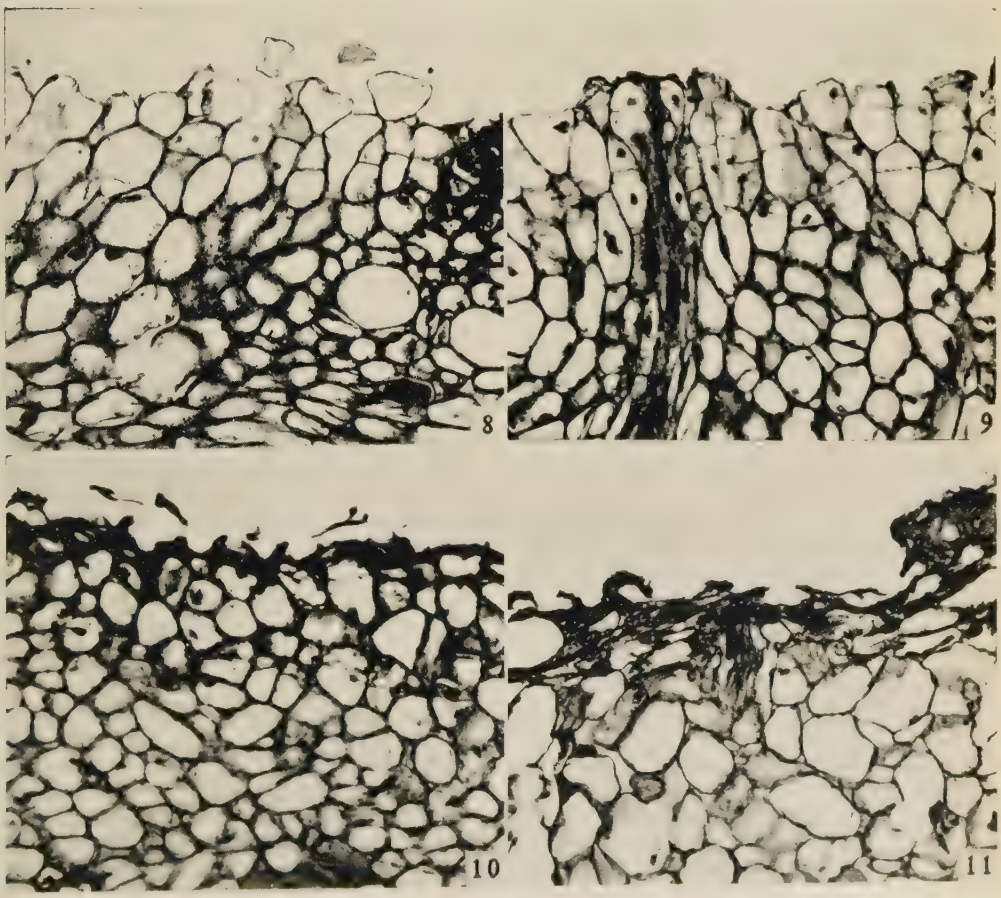
The next change involves the migration of the nucleus to a central position and the accumulation of dextrin-like grains around it (Fig. 6). Although this stage may occur as early as 36 hrs. before separation starts, it is usually preceded by at least 24 hrs. by cell wall alteration in the hypodermis. The nuclei and cytoplasm of the flaccid perianth segments lose the magenta colour reaction characteristic of living cells and stain bright red. In the cytoplasm the characteristic fibril network breaks and becomes granular.

These are followed by cell division, which occurs in late afternoon and evening. Collections made from 6 p.m. to midnight show cells of the abscission layer in active division, mitotic figures, cell plates, and newly completed cell walls being present. Collections made from 6 a.m. to 3 p.m. show no signs of cell division in the abscission layer. Cell division starts in the lower segments and proceeds upward, starting, in each segment, at the top of the abscission zone and proceeding downward. In collections made at 6 p.m., mitotic figures were observed in the abscission layer of the lower or outer segments (Fig. 6), but not in the inner or upper segments. In collections made at 8 p.m. and 10 p.m., mitotic figures were observed in all segments. The figures in the lower segments were generally in a later phase than in the upper segments. Collections made at midnight showed the figures in the upper segments to be in a late phase. Although the lower segments could not be retained, examination of the abscission layer shows it to be intact and with a minimum of injury to the cells (Fig. 9). Also, there are a large number of new cell walls and nuclei in telophase. The walls laid down stain intense green and are generally similar to walls found in the terminal meristem. They connect with the old walls at right angles. The cell divisions are usually restricted to an irregular plate of cells across the base of the perianth segments that form the distal layer of the abscission zone (Fig. 2). However, they may occur in the adjacent layer of cells or, less frequently, in both layers. Infrequently, division occurs in isolated cells deeper in the abscission zone. All the cells in the plane of division do not divide. In the vascular cylinder, division may occur in sieve elements and cells of the sheath.

Concurrent with cell division, rapid changes occur in the cell wall. The first noticeable change is an undulated condition of the walls along the line of probable separation. This usually does not involve a single layer of cells but may simply occur along the wall dividing two cells. It proceeds downward in the same manner as cell division. Such undulations do not occur in adjacent tissue. Simultaneously separations occur irregularly in these walls (Fig. 6). These observations indicate a softening and splitting of the wall. A comparison of the cell walls in the abscission layer with those in adjacent tissues indicates that there is no measurable swelling in this area. Furthermore, the splits do not occur down the middle of the cell wall, but to one side or the other of the region generally defined as the middle lamella. In some places there is a double split, with the middle portion apparently representing the middle la-

mella. The staining reactions of the walls in this area are identical with those in other parenchyma cells.

During cell division and cell wall splitting separation occurs, apparently as a result of the splitting of the cell walls. This usually occurs between the layer of dividing cells and the perianth segment. Infrequently separation may occur along the plane of division of a dividing cell if the wall has been laid down, or it may go deeper into the abscission zone, especially between the dividing cell and the adjacent inner cell. This irregularity concerning position of dividing cells and separation appears to be the result of a straightening out of the separation line in areas where the line of dividing cells is highly irregular. Separation generally proceeds from the top to the bottom of the abscission zone. The cells are left intact by the separation process that occurs as a result of the splitting of cell walls (Fig. 8). However, the perianth segments usually abscise before cell wall splitting is complete and, as a result, there are areas, especially near



FIGS. 8-11. Sections through primary protective layers in various stages of development. × 260. Fig. 8. Surface immediately after perianth segment abscission, showing surface cells intact and deposition of red granules below exposed surface. Fig. 9. Surface immediately after perianth segment abscission in area where cell wall splitting was not completed prior to separation. Note torn cells. Fig. 10. Primary protective layer about 1 week after abscission, showing zone of collapsed cells and zone of altered cells. Fig. 11. Primary protective layer formed over injury to perianth segment. Note starch grains in collapsed cells.

the bottom of the segments, where cells are torn during the process (Fig. 9).

Formation of a Primary Protective Layer

The protective layer formed over the surfaces exposed by perianth abscission was examined and compared with a protective layer formed over a mechanically injured surface of the perianth segment.

To examine a protective layer formed by abscission, a receptacle in which perianth abscission occurred several days previously was collected and fixed at midnight. A primary protective layer had been formed by the collapsing of the outer 2-4 tiers of cells. No evidence of cell division was found in this region (Fig. 10). Beneath the primary protective layer there is a zone of altered parenchyma tissue, 2-3 cells deep. These cells retain their original size and shape (Fig. 10) but the cell walls stain red, indicating suberin deposition. Characteristic cytoplasm is absent and the contents of the cells are crystalline and grey-green, indicating probable death of the cells. In addition, the cells contain numerous red granules characteristic of areas in which protective layers are being laid down. This layer ends abruptly and is adjacent to the normal living parenchyma.

To examine a primary protective layer over an injured surface in this region, the perianth segments of a flower in the process of opening were cut off 1 cm. from their point of attachment. Material was collected periodically and examined in the same manner as previous tissue. Substantially the flower developed normally and cytological phenomena previously described occurred in the same manner as in flowers with uninjured segments. However, these exceptions were noted. A primary protective layer up to 150 μ in thickness is formed by the progressive collapsing of the cells near the injured surface. This layer consists of cell walls with suberin deposited on their inner surfaces and cell cavities, visible as narrow slits. It contains previously formed starch grains, which are absent from the rest of the segment during later stages of development. Red granules, typical of such areas, are present in these and adjacent cells. The injured segments abscised normally. The receptacle was fixed and examined. The abscission zone was not different from that found in normal flowers.

Discussion

Certain observations reported here do not entirely agree with most previous accounts. A review of the literature on abscission shows that abscission zones found in connection with both leaf and petal fall are histologically part of the organ involved (Esau, 1950). In *Magnolia*, the abscission zone at the base of the perianth segment (1) is histologically identical with the cortex; (2) has a starch pattern that, although distinct, is similar to the rest of the cortex while completely different from that of the perianth segment; and (3) just prior to abscission, has a deposit of dextrin-like grains that are absent in the perianth segments. These observations indicate that in *Magnolia* the abscission zone is part of the cortex rather than part of the perianth segment, being different from the types of abscission zones previously reported.

Considerable emphasis has been placed on the relationship between cells of the abscission zone and meristematic cells. In the abscission zone of the perianth segment of *Magnolia*, the cells are of the same size as adjacent cortical cells, have large vacuoles, and show no addition of cytoplasm. In these respects they have features of the normal cortical parenchyma. However, the cell walls of the abscission layer are plastic, the nucleus is centrally located with dextrin-like grains clustered around it, and cell division occurs. In these respects they have reverted to a meristematic condition, but it is not the retention of a meristematic condition as reported by Kendall (1918), Yampolsky (1934), Nightingale and Farnham (1936), and McCown (1943).

Dissolution of the middle lamella has been demonstrated in the abscission of flowers by Kendall (1918) and Yampolsky (1934). Swelling and dissolution of the middle lamella have been shown

to occur in the abscission of flowers by McCown (1943) and Nightingale and Farnham (1936). In *Magnolia* the walls of the cells in the abscission zone and layer show cellulose and cutin patterns identical with the cells of the adjoining cortex, and there is no evidence of swelling when compared with the normal parenchyma cells. The principal changes noted were the wavy or undulated condition and the splitting of the wall. This splitting occurs at various depths and is not necessarily associated with any one layer of the cell wall or the middle lamella. The presence of new cell walls in all stages of development in the abscission layer, in direct contact with walls that are splitting, indicates the presence of materials forming the initial middle lamella. The lack of obvious dissolution of the middle lamella or other wall structures and the lack of swelling is contrary to the majority of other observations on abscission.

The idea that cell division, so frequently observed in connection with abscission, is a separate and distinct process connected primarily with periderm formation rather than with separation has gained momentum. Recently a series of observations on leaf fall was made to support this idea by Gawadi and Avery (1950). In *Magnolia* cell division is concurrent with separation, which occurs in the plane of dividing cells. Further, it has been shown that cell division does not occur in connection with formation of the protective layer over the exposed surface due either to abscission or injury. This indicates that the observed cell division probably has no causal relation to the formation of the protective layer, but is either a causal agent or a result of a common stimulus of separation. Furthermore, the cell divisions in the abscission zone appear to have no function in connection with any other observed phenomena. These observations show that the condition in abscission of perianth segments in *Magnolia* is not in complete agreement with the general statements made by Gawadi and Avery (1950).

Summary

Cytological changes associated with perianth segment abscission were studied under field conditions. Conant's quadruple stain resulted in certain colour patterns upon which the observations in this paper are based. A brief description of tissues involved in abscission is given. The abscission zone is histologically identical with adjacent cells of the receptacle but acts as a separate physiological unit. The phenomena, in order of occurrence, connected with abscission are as follows: (1) splitting of cell walls of the hypodermal cells in the abscission zone; (2) migration of nuclei of parenchyma cells of the abscission zone to the centre of the cells and deposition of dextrin-like grains in these cells; (3) cell wall splitting and cell division in the separation layer; and (4) separation, usually occurring during cell wall splitting and cell division. There is no evidence for dissolution of the middle lamella. A primary protective layer is formed over the surface exposed by abscission that is similar to one formed over an injured surface in the same region. Cell division is not part of the formation of either layer. Observations on the meristematic condition, relationship between the abscission zone and the cortex, cell division, wall structure, and changes in the cells of the abscission zone are discussed.

The writer wishes to express his sincerest appreciation to Professor Arthur W. Haupt, who made this study possible and under whose direction the investigation was conducted. He is also indebted to Professor Fredrick T. Addicott for valuable suggestions and criticisms.

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THE RELATIONSHIP OF ILLUMINATION TO THE DIFFERENTIATION OF A MORPHOLOGICALLY SPECIALIZED ENDODERMIS IN THE AXIS OF POTATO, *SOLANUM TUBEROSUM* L.

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Introduction

The axes of many plants have a specialized sheath of cells surrounding the vascular tissue and variously known as the bundle sheath, starch sheath, or endodermis. This tissue has been frequently described by morphologists and anatomists, who have attributed to it many diverse functions. On the other hand, there have been relatively few experimental studies of the tissue. Quite recently Van Fleet has published a series of important papers (1942a, 1942b, 1943a, 1943b, 1945, 1946, 1947, 1948, 1950a, 1950b) which show the bundle sheath, starch sheath, and endodermis to contain enzyme systems which distinguish them from other tissues, even when there is no structural distinction apparent. He has suggested re-defining the tissue on a physiological basis, and considers the bundle sheath, starch sheath, and endodermis to be different structural expressions of what is fundamentally the same tissue.

The concepts currently prevailing concerning this tissue have recently been reviewed and summarized by Esau (1953) and for the most part need not be duplicated here. Esau has suggested that the term *endodermis* be restricted to those cells of this tissue which have Casparian strips or other types of suberized wall layers, and that the term *endodermoid cell* be used for cells occupying similar position, lacking such wall modifications, but showing some other structural or histochemical resemblance to the true endodermis. This terminology will be used throughout the present paper.

One of the most constant characteristics of the endodermoid and endodermal cell layer is the lack of penetrating air spaces or intercellular spaces between the cells of this layer and those to either side of it (Artschwager, 1918, 1924, 1925; Eames &

MacDaniels, 1947; Haberlandt, 1928; Hayward, 1938; Priestley & Swingle, 1929; Scott & Priestley, 1928). This feature prevents the exchange of elements in a gaseous state between the cortex and stele, and may aid in preventing air from entering the conducting tissues (Priestley & North, 1922).

In the root, and to a lesser extent in the stems of Angiosperms, the modifications of the walls of endodermal cells with suberin, cellulose, and lignin have lead to much discussion of the possible functions of the endodermis. It has frequently been assumed that suberin, which is known to be relatively impermeable to both water and gases, when deposited on the radial and tangential walls of endodermal cells in the familiar Casparian strips, aids in maintaining higher osmotic pressure in the conducting tissue by preventing radial passage of water or of solutes through the endodermis via the cell wall, and all such passage of water or solutes through the endodermis is thus placed under the control of the protoplast of the endodermal cells (Priestley & North, 1922).

Priestley and his students have published a series of papers relating to the development of the modifications of the walls of endodermal cells under varying environmental conditions. The papers of this series most significant to the present investigation suggest that the formation of Casparian strips in the endodermis may be explained by the movement of fatty substances outward from the stele; when these substances enter the endodermoid layer, with its higher supply of oxygen along its outer face, the fats are presumably in part oxidized to form the Casparian strips (Priestley & North, 1922; Lee & Priestley, 1924). It is also suggested (Priestley & Ewing, 1923) that in the aerial axes of broad bean, *Vicia faba* L., potato, *Solanum tuberosum* L., and other plants which show similar types of etiolation, this mechanism only takes place in the absence of light. The results of experiments are described (Priestley, 1922; Priestley & Ewing, 1923) in which potato and other plants were grown in darkness, and under these conditions a typical starch sheath was first formed in the aerial axes; the starch slowly disappeared from the sheath, and suberized Casparian strips were deposited in the cells. Transition from a starch sheath to endodermis in the etiolated shoot progressed in an acropetal direction, and, at the region of transition, cells showed both Casparian strips and a few starch grains (Priestley, 1926). These results imply that, in the aerial axes of many Angiosperms, exposure to light inhibits endodermoid cells from differentiating into an endodermis. Unfortunately, none of the published reports of the work just described contain more than passing references to the conditions under which the experimentation was performed.

The axial anatomy of four varieties of *Solanum tuberosum* has been studied in great detail by Artschwager (1918). Both field-grown and greenhouse-grown potatoes were included. Artschwager found that the aerial stem, when the leaves were nearly mature, contained an endodermis composed of a single layer of cells which differed from the adjacent cortical cells in their smaller size, more regular arrangement, and lack of intercellular spaces. "Casparian strips are present, but the lignified area is not always noticeable." The endodermal cells were described as containing some starch, even when all other tissue is empty of starch, and the starch content is "in some (potato) plants the most constant criterion for the identification of the endodermis, since the Casparian strips are not always distinguishable". In the stolons the endodermis was found to contain much less starch than in the other tissues; this observation was a modification of that of Reed (1910), who described the endodermis of the potato stolon as free from starch, even when the surrounding cortical tissue was crowded with starch grains.

From even casual comparison between the findings of Priestley and his students and the findings of Artschwager on the aerial light-grown potato stem, it is apparent that some question can be raised as to whether or not illumination is a causative factor in determining whether a true endodermis with Casparian strips, or an endodermoid layer in the form of a starch

sheath, will be differentiated in the axis of potato. The present paper describes the results of experiments designed to test the influence of light on structural expression in this tissue, and data are presented which suggest that illumination has little if any direct effect on the differentiation of the tissue.

Materials and Methods

Four series of plants were grown for this study:

I. *Controls* — Plants grown under more or less normal conditions of illumination, which received a 12-hour period of illumination each day.

II. *Etiolated* — Plants grown in complete darkness, for the duration of the experiment.

III. *Dark to Light* — Plants at first grown in complete darkness, and then transferred to the conditions of the control series.

IV. *Light to Dark* — Plants first grown under the conditions of the control series, then transferred to complete darkness.

The conditions under which the experiment was conducted and the methods employed are described in some detail to enable other investigators to duplicate or compare them.

Six unglazed earthenware flowerpots 8 in. in diameter were filled within half an inch of the brim with a mixture of two parts loam soil to one part sand. Four "eyes" from Irish Cobbler potato tubers of the same seed lot were planted in each pot, 1½ in. beneath the surface of the soil, and spaced laterally approximately equidistant from each other and from the edge of the pot. The experiment was conducted in a greenhouse under conditions of temperature, humidity, and soil moisture which were comparable for all of the plants involved. The daily illumination period was extended to approximately 12 hours for all series of plants being grown in the light by burning six 200-watt clear glass incandescent bulbs suspended 43 in. above the tops of the pots, from 4.30 p.m. until 7.00 p.m. Temperatures ranged from 65°F. night to 75°F. day, but occasionally would rise to 78°F. on clear sunny days. The soil in all pots was kept moist to the touch but not wet.

Two of the six pots of plants were allowed to develop in the light under the conditions described above, and served as control plants. The four remaining pots of plants were allowed at first to develop in total darkness. This was accomplished by placing over the pots a double-walled corrugated pasteboard box which was 2 ft. wide, 3 ft. long and 2 ft. high; the lower edges of the box were buried in the drainage gravel on the tray of the greenhouse bench to prevent any light from entering. The outside of the box was in turn covered with a black cotton cloth to insure complete darkness inside. When the box was removed briefly from time to time to obtain samples of the etiolated plants for study, great care was taken to insure that the plants did not receive any illumination while uncovered.

The tubers had sprouted and shoots emerged from the soil within 9 days after planting. Samples from both the light-grown and the etiolated series were collected 19 and 24 days after planting, and freehand transverse and longitudinal sections made of comparable regions of the axis. Sections were tested histochemically: the presence of lignin was determined by the phloroglucin — hydrochloric acid reaction[1]; fats and suberin by Sudan IV[2]; and starch by iodine — potassium iodide[3]. For general anatomical study of the samples, Delafield's hematoxylin, aniline blue, and neutral red were employed. Of these, a 0·1 per cent aqueous solution of neutral red gave the most satisfactory results.

After 26 days, one of the two pots of the control plants was placed under the box in the dark, and two pots of the etiolated plants were transferred to the bench

1. Sections treated with several drops of 0·1 g. phloroglucin dissolved in 95 per cent ethyl alcohol, followed by a drop of 25 per cent HCl.

2. Sections treated for 20 minutes with a solution of 0·5 g. Sudan IV dissolved in 100 c.c. 70 per cent ethyl alcohol; then transferred to glycerin for study.

3. Sections treated for 5 minutes with a solution of 0·3 g. iodine and 1·5 g. potassium iodide dissolved in 100 c.c. distilled water; then coverslipped.

outside the box. The two other pots remained in the dark. Samples were taken from these four series 7 and 17 days later, and were studied anatomically and tested histochemically for starch, fats, suberin, and lignin. The experiment was discontinued after a total of 43 days.

Observations

First Sampling (19 Days After Planting)

The control plants were at this time from 6 to 8 in. high, green with normal

TABLE 1 — SUMMARY OF DATA FROM SAMPLINGS

Time	Series	Portion of plant	Substances in endodermoid cells	External phloem fibres	Internal phloem fibres	Substances in cells other than the endodermoid sheath
19 days	I	1st, 3rd, 4th aerial internodes	Starch	—	—	Starch; fats
	II	1st, 3rd, 4th aerial internodes	—	Yes	—	Fats
24 days	I	Subaerial internodes	—	Yes	—	Starch; fats
		1st, 3rd, 5th aerial internodes	Starch	—	—	Starch; fats
	II	1st, 3rd, 5th aerial internodes; and subaerial internodes	—	Yes	—	Fats
33 days	I	Roots	—	Yes	—	Fats.
		Subaerial internodes	—	Yes	Yes	Starch; fats
		1st, 3rd, 5th, 7th, 9th aerial internodes	Starch, chlorophyll	Yes	Yes	Starch; fats
	II	Roots	—	Yes	—	Fats
		1st, 3rd, 5th, 7th, 9th aerial, and subaerial internodes	—	Yes	Yes	Fats
	III	Roots	—	Yes	—	Fats
		1st, 3rd, 5th, 7th, 9th aerial internodes	—	Yes	Yes	Fats
	IV	1st, 3rd, 5th, 7th, 9th aerial internodes	Chlorophyll	Yes	Yes	Fats
		18th aerial internode (developed in the dark)	—	Yes	Yes	Fats
43 days	I	1st, 3rd, 5th, 7th, 9th, 11th, 13th aerial internodes	Starch, chlorophyll	Yes	Yes	Starch; fats
	II	1st, 3rd, 5th, 7th, 9th, 11th, 13th aerial internodes	—	Yes	Yes	Fats
	III	1st, 3rd, 5th, 7th, 9th, 11th, 13th aerial internodes	—	Yes	Yes	Fats; starch
	IV	1st, 3rd, 5th, 7th, 9th, 11th, 13th aerial internodes	Chlorophyll	Yes	Yes	Fats
		Aerial internodes developed in the dark	—	Yes	Yes	Fats

Series I: Controls, grown in a 12-hour photoperiod.
Series II: Etiolated plants, grown in continuous darkness.
Series III: Plants transferred from dark to light (26 days in dark, then transferred).
Series IV: Plants transferred from light to dark (26 days in light, then transferred).

young foliage. The etiolated plants were from 12 to 14 in. high, pale pinkish-white, with greatly elongated internodes and rudimentary leaves, and presented the appearance of etiolated potato stems as described by Priestley and Ewing (1923). Two stems from each series were sampled, and the data from this and from subsequent samplings are recorded in tabular form for comparison in Table 1.

Series I: *Control Plants* — Sections from the first internode above ground, and subsequent internodes showed the endodermoid layer to be a typical starch sheath. These endodermoid cells were regularly arranged, lacked penetrating intercellular air spaces, and contained a much larger proportion of starch plastids than did the surrounding tissues of the cortex. There was no lignification or suberization of the walls of these cells. Fats were present in the stem, but could not be detected in the endodermoid cells. Scattered fat idioblasts were observed in the cortex, and the epidermal cells had a well-developed cuticular layer. Samples of this series of plants showed that all of the starch in the stem was converted into soluble substances during the day, including the starch in the starch sheath. Samples taken shortly after 7.00 p.m. always showed the large deposit of starch in the sheath (Fig. 1).

Series II: *Etiolated Plants* — On the whole the anatomy of these stems differed only slightly from the series grown in the light, but the stems contained no starch, and were completely devoid of chlorophyll. The endodermoid cells could be distinguished on the basis of position and cell arrangement, which was similar to that in the control plants. There was no lignification or suberization of the walls of the cells of this tissue in any of the internodes, although fat idioblasts were found in the cortex, and the epidermis of these stems also had a well-defined cuticle. The major anatomical difference was seen in the phloem just to the inside of the endodermoid layer, where long fibres with cellulose wall thickenings had been differentiated. These elements were differentiated upward almost to the growing point, and were thick-walled, with the cell contents apparently alive in the lumens (Fig. 2). The cellulose walls of the parenchymatous tissues were much thinner in these plants than in the controls.

FIGURE 1.

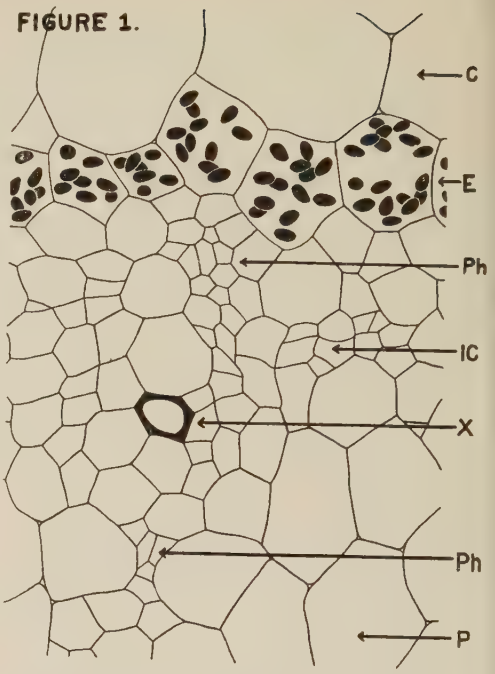

FIG. 1 — Transverse section of stem, third internode above the surface of the soil, control plant, interfascicular vascular region, showing the differentiation of the tissues 19 days after planting. × 150. (*C*, cortical parenchyma; *E*, endodermoid sheath, with starch plastids; *Ph*, phloem; *IC*, interfascicular cambium; *X*, xylem; *P*, pith.)

SECOND SAMPLING (24 DAYS AFTER PLANTING)

Series I: *Control Plants* — The aerial portions of the stem were as previously described. No modifications of the walls of the endodermoid cells were visible, and the tissue was again found to contain much starch at night, none during the day. Fat idioblasts were now in the pith as well as in the cortex. No phloem fibres had developed. Underground portions of the axis more nearly approximated the etiolated aerial stems of Series II in structure. Phloem fibres in the external phloem were as numerous, and

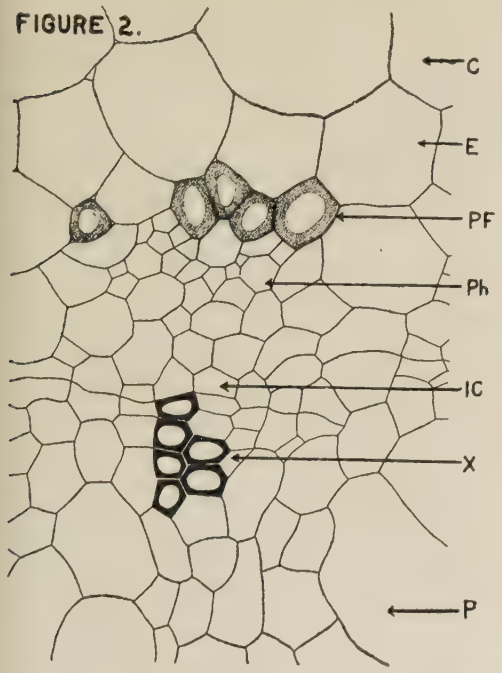

FIG. 2 — Transverse section of stem, third internode above the surface of the soil, etiolated plant, interfascicular vascular region, showing the differentiation of the tissues 19 days after planting. × 150. (*C*, cortical parenchyma; *E*, endodermoid sheath; *PF*, phloem fibres; *Ph*, phloem; *IC*, interfascicular cambium; *X*, xylem; *P*, pith.) Note the lack of starch plastids in the endodermoid sheath cells, the large well-differentiated fibres in the external phloem, and the relatively large amount of secondary xylem, as compared with the control plant.

although the cortex contained considerable amounts of starch, no starch was found in the endodermoid cells, either during the day or at night. No modifications of the walls of this tissue were discernible.

Series II: *Etiolated plants* were as already described in the previous sampling.

Plants in both Series I and II initiated at ground level a number of adventitious roots. Sections of this region showed cells of the stem cortex bordering the epidermis of the growing root with a suberized band on the walls parallel to the developing root. It was determined that these cortical cells with the suberin deposits were not extensions of the endodermoid cylinder of the stem, but were merely ordinary cortical parenchyma cells which had developed this type of wall structure, suggestive of an endodermis with secondary thickenings, apparently as a response to the penetration of the tissue by the developing root.

THIRD SAMPLING (33 DAYS AFTER PLANTING)

Series I: *Control Plants* — Phloem fibres had been differentiated both in the external and internal phloem of the aerial stem. The endodermoid cells were still a starch sheath, but also contained considerable chlorophyll. Chlorophyll and starch were contained in the same plastid; during the day only chlorophyll was seen in the plastids of the sheath; at night both starch and chlorophyll were present in each plastid. There was considerably more chlorophyll concentrated in the cells of the endodermoid layer than in the cells of the cortical parenchyma, and it appeared to contain as high a chlorophyll content as the epidermis of the stem. The sheath was easily distinguishable from adjacent tissues at this sampling because of this feature.

Series II: *Etiolated plants* had continued to elongate and to differentiate additional nodes and rudimentary leaves, but anatomically remained much as previously described. Phloem fibres had differentiated in the internal phloem. No starch or chlorophyll was detectable in these plants. No wall modifications of the endodermoid layer could be observed.

Series III: *Plants transferred from dark to light* (26 *days in dark*, 7 *days in light*) had developed considerable chlorophyll in the leaves and a small amount in the epidermis of the stem, but did not have any stored starch in the stem during day or night. The endodermoid cells remained unchanged from their condition as described for etiolated plants.

Series IV: *Plants transferred from light to dark* (26 *days in light*, 7 *days in dark*) retained the chlorophyll in the endodermoid cells in those portions of the stem which had developed in the light, but contained almost no stored starch. Other axial tissues were completely free of stored

starch. New nodes and internodes which had been differentiated after transfer to the dark were as etiolated and showed a structure comparable to plants grown in constant darkness. These regions had no chlorphyll or starch in the endodermoid or other tissues.

Fourth Sampling (43 Days After Planting)

Series I: *Control Plants* — Tissues were as described from the third sample. No modifications of endodermoid cell walls.

Series II: *Etiolated Plants* — Tissues as described from the third sample. No modifications of endodermoid cell walls.

Series III: *Plants Transferred from Dark to Light* (26 *Days in Dark*, 17 *Days in Light*) — Starch now present at night in the axes, but none in the endodermoid sheath, which remained as it was while these plants were in darkness. The endodermoid cells in internodes which had been differentiated after the plants were transferred to the light resembled those in the internodes which had developed in the dark, and were devoid of both chlorophyll and starch, although neighbouring tissues showed both of these substances in quantity.

Series IV: *Plants Transferred from Light to Dark* (26 *Days in Light*, 17 *Days in the Dark*) — These plants had lost all foliage which had been formed while growing in light, and the axes no longer gave a positive starch reaction in any of the tissues. Some chlorophyll remained in endodermoid cells in those portions of the axes which had been formed while exposed to light. No wall modifications of endodermoid cells could be detected throughout the axes.

At this time, the plants remaining in all four series were dug up and the roots were sectioned and studied. None of the roots examined had developed a typical endodermis with Casparian strips or other types of wall modifications. Underground portions of the stolons from all four series were also examined, and were found to be similar to the aerial axes of etiolated plants, in that the endodermoid cell layer lacked starch and the walls were unmodified.

Summary and Conclusions

In the potato, *Solanum tuberosum* L., under the conditions and duration of this experiment:

1. An endodermoid cell layer is differentiated between the cortex and stele throughout the axis, and is visually recognizable in sections of the axis on the basis of position, cell size, shape, and arrangement. In the internodes this tissue forms a solid cylinder which encases the stele, and it is not penetrated by intercellular spaces.

2. No Casparian strips or other wall modifications requisite to classify this tissue as an endodermis are developed in either aerial or subterranean portions of the axis, whether the plants be grown in normal light, grown in continuous darkness, or shifted during their development from one of these two environments to the other.

3. In the subterranean portions of plants grown in normal light, and in all portions of plants grown in continuous darkness, the cell walls of endodermoid tissue remain parenchymatous and do not include lignin or suberin; the protoplasts contain fewer fats than in the cells of either the cortex or pith, and they never contain starch.

4. In the aerial portions of young plants grown in normal light conditions, the endodermoid layer is first a typical starch sheath, but contains starch only during the night. It subsequently becomes a chlorophyllous sheath as well.

5. In plants transferred from light to complete darkness, the starch content of the layer disappears, and the chlorophyll tends to disappear.

6. In plants transferred from complete darkness to light, the majority of the tissues in the stem tend to become similar to those of the light-grown plants; and after a time contain chlorophyll and stored starch, but the endodermoid layer does not become either a starch or chlorophyllous sheath, and remains in much the same condition as when developed in the dark. This condition of the endodermoid cells persists in those portions of the axis differentiated after the plant has been transferred to normal light conditions.

7. Illumination tends to retard differentiation of the phloem fibres.

8. The development of a typical endodermis, with Casparian strips or other modifications of the cell walls, is dependent on factors other than illumination, lack of illumination, or the presence of fats in the tissues. Exposure to light during early growth appears to be necessary for the development of a starch or chlorophyll sheath in the endodermoid layer.

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PLEIOCOTYLY AND DIFFERENTIATION WITHIN ANGIOSPERMS

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Historical Introduction

The phenomenon of extra cotyledons in both the Dicotyledons and Coniferae, called Pleiocotyly (Gk.: pleion: more; cotyledon: seed-leaf), has often been recorded in the literature. Under this term monocotyly as well as polycotyly are included. The following is a brief review of previous work, emphasizing only those authors who have realized the wider implications of this phenomenon.

Lubbock (1896) in his classical work on seedlings lists the frequency with which tricotyledonous types occurred, mostly reported by Gruppy (1895). Besides purely tricot seedlings, Lubbock noted that many species have one or both cotyledons divided, e.g. *Primula sinensis* seedlings often have one of the cotyledons deeply bifid. *Peucedanum sativum* seedlings often have one or both of the cotyledons bifid, bipartite or divided to the base, i.e. simulating three or four cotyledons instead of two. Where fission occurred there was a distinct midrib to each lobe of the cotyledon. In *Opuntia basilaris* besides one of the two cotyledons sometimes being bifid, only one cotyledon occasionally was present. Finally Lubbock, quoting Gruppy, records that abnormalities occur in from 2 to 6 per cent of the seedlings observed in the British plants.

Anatomical studies of pleiocot seedlings in a number of species, e.g. *Cheiranthus* and *Impatiens*, were made by Holden and Bexon (1918), Holden (1920), Holden and Daniels (1921) and Bexon (1926) in attempts to establish the true relationship of polycotyly.

Brozek (1926) using three species of *Mimulus* found that although the condition was inherited he was unable to find in *quinquevulnerus* lines the method of inheritance of seedlings with cotyledons abnormally grown together. Saunders (1928), in the course of her work with *Matthiola incana*, also studied syncotyly and tricotyly, the former being more frequent in this species. She believed neither condition was inherited, and observed also that the frequency of both varied from year to year; larger numbers of syncotylous seedlings occurred from seed ripened under good conditions. This she studied with the idea that syncotyly may arise indirectly as the result of a certain misfitting of the developing embryo to the mould of the embryo sac.

Munerati and Costa (1934), in their studies of teratological forms of *Beta vulgaris* found that it was not possible to stabilize for extra cotyledons, although they found that the number increased as a result of cross-breeding. They found that there was a correlation between cotyledon and root structure, dicots being diarch and tricots triarch. Tucker (1938) drew attention to the fact that varieties of apples which have more than five carpels in some fruits often have tricot and tetracot seedlings, compared with penta-carpellous varieties which normally produce only dicots. He found that tetracots occurred more frequently with increase in carpel number of the fruit. Different varieties have the same trends but at different rates. Tucker suggested that parallel trends between parent tissue (fruits) and offspring tissue (seeds) might indicate that the behaviour was caused by the same condition.

Litovchenko (1940) discussed reasons why cotyledons had been reduced in number during evolution, suggesting, for instance, that a reduced number of coty-

ledons was beneficial by reducing evaporating surface. He considered tricots very important as they represent a group of high productivity; they possess new biological and economic properties resulting in higher activity and in economic advantages. Tricots in sugar-beet occurred mainly among the larger seed clusters. Like De Vries (1910) he found that the phyllotaxy was different for tricots and dicots, leaves of dicots being multiples of two and of tricots being multiples of three. In tricot roots there are three orthosticha of feeding roots instead of the normal two. Litovchenko (1940) considered that higher root number helped in nutrition; hence tricots should give a better yield. He found anatomical conditions similar to those found by Munerati and Costa (1934), and considered tricots were " improved " anatomically and should, therefore, have, indirectly, higher yields and higher sugar content than dicots. Most of Litovchenko's results are marred by omission of some important points in his statistical presentation and it seems that in many instances his conclusions rest upon the comparison of one plant with another. He found that tricot castor oil plants were more vigorous and fertile than dicots. In general, he considered tricots to be of great economic value; they probably arose through mutation under the conditions of abundant nutrition. He suggested that Soviet agriculture would benefit from the use of tricotyly.

Pleiocots are not confined to diploids. Noguti, Oka and Otuka (1940) made tetraploids of *Nicotiana tabacum* and obtained a few seedlings with one or three cotyledons, the dicots prevailing. Miss Janaki-Ammal (private communication) has noticed in her experiments on artificial tetraploidy in garden flowers that tetraploids have just as many pleiocots as dicots from which they are derived; in some species, e.g. *Nicandra*, they are more frequent in tetraploids. Although her data are only provisional, it is suggested that pleiocotyly is, in some way, associated with a state of unbalance of the chromosomes. Leandri (1941) examined mitoses in a tricot seedling of haricot bean and found no chromosomal differences from normal except that chromosomes of the tricot were slightly shorter than those of normal seedlings.

Barnes (1943) studied pleiocotyly in *Anagallis arvensis* L. and found a range between true dicotyly and tricotyly. Some of the tricots had fused epicotyledonary leaves and, although tricots had a third more assimilating tissue than dicots, they lagged behind the dicots in development, probably through lack of vigour. This is probably true also in *Antirrhinum majus*. Barnes commented: " The emphasis which, rightly, has been placed on the importance of dicotyly in the flowering plants, seems to have engendered a reluctance to admit that meristic variation may effect even this deep-seated character, and to have favoured explanations based on suggestions of injury to growing points or of the splitting of rudiments." From comparison of healthy normal dicots and tricots one cannot tell whether there has been splitting during ontogeny or the rudiments arose and developed independently. It might in future be shown that dicots are better balanced in general organization than are tricotyls. Although a greater assimilating surface should help a seedling to survive competition, however, " occurrence of tricotyly in many families of dicotyledons, not all closely related, may be an experiment in evolution, which, if it succeeds, will make dicotyly a less important character than it is now ".

Went (1944) made observations on pleiocotyly in the tomato varieties " San Jose Canne " and " Marglobe " (see Fig. 7). Syncotyly was rare (0·1 per cent) and tricotyly (0·0024 per cent) rarer than in most plants. Tricotyls have three first leaves instead of two; those developed simultaneously were equal sized and practically whorled alternating with the three cotyledons. One such plant had its main stem bifurcated after the third foliar whorl; both branches had normal spirals in opposite directions. Went linked up tricotyly with fasciation. If such a condition arose through enlargement of the growing point so that three instead of two cotyledons can be initiated simultaneously in a whorl, this enlarged growing point persists for varying periods afterwards. Hence first leaves also form in a whorl of

FIGS. 1-7 — Pleiocotyly in Clucas 99 Tomato. Fig. 1. Dicot. Fig. 2. Dicot — abnormal. Fig. 3. Pseudo-tricot. Fig. 4. Tricot (right-angled). Fig. 5. Tricot (equi-angled). Fig. 6. Pseudo-tetracot. Fig. 7. Tetracot. ×2/3.

three. Then either the size of the growing point decreases to normal giving rise to normal 2/5 phyllotaxy or the whorled condition persists until the enlarged growing point splits into two with true dichotomy. A pleiocotylous series in the tomato is given in Figs. 1-7.

Holtorp (1944) has re-focussed attention on the genetical basis of tricotyly. By growing tricot brassicas in isolation for two generations, he got a marked increase in tricotyly frequency and also obtained quantities of tetracots, thus demonstrating a genetical control. He considered that dicotyledony must be adaptive and maintained by natural selection. Holtorp analysed pleiocots in brassicas into three groups: (a) *simple fission*, i.e. with a split cotyledon; this is always distorted and slower growing than the other cotyledon; (b) *twinning*, e.g. a two-notched broad cotyledon with a prominent central peak and forked midvein, and is the only type occurring in both cotyledons; his diagram showed various stages towards true tricotyly; and (c) *supplementation*: this is common in progeny of tricots after selection and includes (i) normal dicotyledonous seedling with the first leaf cotyledonous of various pleiotropic grades from leaf to cotyledon; (ii) a third cotyledon instead of a second epicotyledonous leaf, the first being normal, but position actually may vary; (iii) the third cotyledon may be borne opposite the first true leaf. Such seedlings grow into good plants. However, fission and supplementation lead to local malformation though twinning does not. Tricot brassicas sometimes bear a pair of true leaves at the first node, or the first and second leaf may be incompletely twinned. Holtorp

noted that in tricots of wallflower, carnation and *Dianthus sinensis* there are often three true leaves at the first and perhaps at the second node. Of the teratological forms obtained by Holtorp (unpublished), interesting floral forms were obtained in *Campanula* species, with flowers having extra sepals, petals and stamens, and stigmas with additional lobes.

In wallflower (*Cheiranthus*) Holtorp obtained a seedling with six cotyledons following selection for pleiocotyly. He noted that at tetracotyly there is a pause before selection can break down this stage but, once broken through, pentacotyls can be obtained.

Holtorp's (1944) experiments with brassicas are open to three criticisms: (1) overcrowding, which leads to bolting and so masking phenotypes that would appear if the plants grew under normal conditions; (2) spatial isolation could not be considered satisfactory; and (3) in some instances there was a deliberate attempt to inbreed, so masking the appearance of recessive genes from recombinations following selection.

Some of Holtorp's tetracot brassicas following selection for pleiocotyly produced white seeds in their pods but, as these did not appear till late in the selections, Holtorp believed this could not be due to recessive genes segregating, as they would have appeared previously. Similar ly he noted the appearance of pods with additional valves. In pleiocotylous *Antirrhinum* flowers he found that there were internal basal growths which pushed open the throat of the flower. Also in pleiocotylous brassicas he often obtained " pseudo-twin " stamens, which he thought might be like those of a related family.

More recently, Straub (1948) has considered both hereditary and environmental factors. He considers there are two or three pairs of genes controlling tricotyledony: tt with or without the presence of Z_1, Z_2; z_1, z_2. Like Saunders (1928), he found that external conditions were important. For instance, if embryos developed in the autumn 20 per cent would be tricots compared with 0·5 per cent developing in summer. If leaves were removed in summer time, there was an increase in tricotyledony and Straub interpreted this as due to lack of nourishment during embryo development. This is an external factor favouring tricot development. White (1948) has recently drawn attention to the phenomena of fasciated and multiple branching types of plants associated with the occurrence of extra cotyledons.

An Evaluation of De Vries' Pleiocotyly Studies

A more detailed résumé with some comments is given below of De Vries' (1910) experiments recorded in *The Mutation Theory*, because full credit is due to him for his careful observations and experimental work. Although many papers reporting pleiocotyly have appeared, few refer to this work. Apparently although *The Mutation Theory* is still referred to by geneticists it is no longer consulted by plant scientists in general. The important conclusions on mutation as a factor in evolution, and the emphasis on *Oenothera* with its exceptional behaviour, led to an overshadowing of his investigations on pleiocotyly.

In addition, in the light of neo-Mendelian inheritance and Mather's (1943) polygene concept for interpreting the inheritance of quantitative characters, it is now difficult to read De Vries and understand some of the difficulties which he experienced. If one disagrees widely with, for instance, his classification of intermediate and half races, this need not detract from the pioneer experimental work which was carried out on a scientific basis. Even if theories come and go, the facts, provided that they have been obtained genuinely, will always outlast the theories.

De Vries (1910) found that some species produce greater numbers of tricots than others. If these variants were rare they were normal tricots; if more numerous then they usually varied in both a plus or minus direction. Deviations become rarer the further they are from tricotyly. He obtained a morphological series in *Oenothera hirtella*. The various forms of pleiocotyly occurred in different proportions, the " hemi " or intermediate stages being rarer than true tricots both

in commercial and selected stocks. De Vries (1910) considered dicotyly as an unvarying character while tricotyly has great variation; dicots which come from tricot parents are "atavists". Unfortunately he did not realize that the series could result from different concentrations of genes controlling a quantitative character. He found tricots commoner among cultivated species or from wild species grown in botanical gardens than amongst wild ones.

De Vries (1910) separated out species into two categories:

(a) *Half Races* — These comprise most species. They throw tricots, but selection of these does not increase their proportion in later generations.

(b) *Intermediate Races* — These also occur in a few species and can be isolated from half races. The difference between them lies in their percentage composition of cotyledon type rather than in actual characters.

Half races and intermediate races do not pass from one to the other by selection. Half races have nearly all dicot offspring, the proportion of individuals with a large number of tricot offspring decreasing rapidly. On the other hand, intermediate races are altered by selection and external conditions. Hemi-tricots tend not to reproduce their own type but behave like tricots; it is thus impossible to "fix" these into a pure line.

De Vries (1910) somewhat mars his discussions by separating races into these two categories. His confusion was caused by influence of his previous conclusions on over-sporting races of other anomalies he had investigated. Possibly also he was confusing inbreeding and outbreeding species. De Vries gives detailed records of a large number of half races which he selected for three years without any definite increased production of pleiocotyly. Except for *Lychnis fulgens* and *Penstemon gentianoides*, the following showed no response: *Oenothera rubrinervis*, *Chenopodium album*, *Polygonum convolvulus*, *Silene conica* and *S. conoidea* and *Spinacia oleracea*. "Thus", De Vries (1910) pointed out, "we see that tricotylous half races exist which even under the most stringent selection can produce only small percentages of the anomaly." He concluded that half races were widely distributed among plants where no more than an occasional aberrant form occurs in 10,000 seedlings, this low frequency as a rule strongly suggesting that the species is a half race. However, as these are generally outcrossing species there should be a selective advance, and I have, therefore, re-analysed De Vries' (1910) data. For five of the species, the mean percentage of tricotyly is given in Table 1 and this may be interpreted as an advance, even though somewhat slow.

TABLE 1 — GENERATION OF SELECTION FOR PLEIOCOTYLY

(*Average of 5 outbreeding species*)

	Generation			
	1	2	3	4
Pleiocotyly, %	1·0	1·4	2·0	1·9

De Vries (1910) also carried out a lengthy experiment with *Amaranthus speciosus* and concluded, after ten generations of selection, that the yearly figures for pleiocotyly "indicates a fluctuating around a constant mean value than a steady progress under the influence of selection". This, according to him, indicates that a half race does not have either a sudden or gradual transition to an intermediate race.

In *Scrophularia nodosa* tricot selection was also practised for several years. Progress was continual, and selection on the whole was successful. But De Vries (1910) considered that any chance outcrossing reduces the chances of pleiocot production. *Amaranthus* and *Scrophularia* were self-pollinated according to him, and so he used the self-fertilized *Oenothera berteriana*. This he found would not produce an intermediate race. Thus De Vries, unknowingly, produced evidence that inbred species may produce pleiocots, but selection has little effect in increasing the proportion of their occurrence (cf. Haskell, 1951). (Actually *Scrophularia nodosa* is proto-gynous so that flowers have a considerable chance of being cross-pollinated by pollen from other plants.)

The increased production of pleiocots by selection of intermediate races was also studied by De Vries (1910). According to him, no artificial pollination is necessary and it takes place as quickly in dioecious wind-fertilized flowers as in self-fertilized species. This is in contrast with results from my experiments (Haskell, 1951).

De Vries (1910) notes that intermediate races, which possess high amounts of pleiocots before selection is commenced, are best obtained from commercial seed. This may perhaps be interpreted as indicating that selection by man for certain peculiarities of adult plants has, by correlation, brought about alterations in the balance of dicotyledony (Haskell, 1954). Thus cotyledon aberrants are more frequent in cultivated species than in wild. This would be a corollary of the correlated responses already reported.

The highest amount of pleiocotyly which De Vries found in a wild species was from a hemi-peloric strain of *Linaria vulgaris*. Both in *Oenothera hirtella* and in *Cannabis sativa* there was a selective advance. The latter is a dioecious species so outcrossing is obligatory. It would be of interest to determine whether selection for pleiocotyly upsets the sex mechanism in this and other species. Increased pleiocotyly was also obtained in *Mercurialis annua*, another dioecious species; also in *Clarkia pulchella* and *Helichrysum bracteatum compositum*, where large quantities of tetracots appeared. De Vries (1910) added: "We may conclude that the tetracotyls are not more likely, nor on the other hand less so, to produce tricotyls than the tricotyls themselves, but that they obviously belong to the same race, i.e. that their character is brought about by the same elementary factor."

TABLE 2 — GENERATION OF SELECTION FOR PLEIOCOTYLY IN *ANTIRRHINUM MAJUS*

	Generation					
	1	2	3	4	5	6
Mean % pleiocotyly	5	6	13	26	50	64

TABLE 3 — DE VRIES' PLEIOCOTYLY SELECTIONS IN *PAPAVER RHOEAS*

18	19	19	20	—	—	—	Selective advance: Intermediate race
12	12	—	—	—	—	—	Hybrids between the two races segregate both races
7	7	8	—	—	—	—	
4	4	4	4	6	—	—	
0·5	1	1	1	1	1	2	Half race No selective advance

Figures refer to percentage tricotyly in 21 seedlings.

With *Antirrhinum majus* a strain was isolated which gradually increased in pleiocotyly (Table 2), while in *Papaver rhoeas*, from 21 tricots the percentage tricotyly obtained is summarized in Table 3. When summing up these selection experiments, De Vries (1910) noted that certain percentages appear much more commonly than others, usually around 55 per cent, and this is usually reached at the conclusion of the second generation or sometimes third or fourth. He compared such effective selection for these intermediate races with those from half races where selection was virtually ineffective and concluded that, "there can be no doubt that entirely different factors are at work in these two cases". The one can be separated from the other as the intermediate race is never found pure by itself.

De Vries (1910) also discussed the influence of tricotyly on leaf arrangement and other plant characters. Plants normally with decussate leaf arrangement may have whorls of three, affecting all leaves or only the lower ones. The production of cleft leaves, twisted and fasciated stems and production of terminal leaves, are all part of the phenomenon. He concluded that these abnormalities have a genetic connection with pleiocotyly. In some species certain abnormalities are more frequent than in others. He was able to produce a strain of *Dracocephalum moldavicum* with a spiral twisting by using this knowledge. He noted, too, that dicots taken from tricotylous cultures sometimes have a ternary leaf arrange-

ment at later stages like some of the tricots but, as in *Asperula azurea*, stem and branch abnormalities are not so frequent (75:27 per cent) in dicots (atavists) as in tricots. He wisely observed that the present case indicates that the internal cause is not necessarily limited in its operation to the cotyledons. Tricotylous races of *Antirrhinum majus* gave specimens with terminal leaves of various grades, e.g. single or double, either fused or funnel-shaped, but De Vries observed with caution that this need not necessarily indicate a causal relation between this character and tricotyly.

De Vries (1910) also devoted a chapter of *The Mutation Theory* to a study of syncotylous races. He listed many species with them and defined them as individuals whose seed leaves are completely, or almost completely, fused together along one side. The cotyledons sometimes even form pitchers. He noted the range through the monocotylous series to the true tricotylous condition but failed, in his discussions, to realize that he was dealing with the gamut of one phenomenon.

In *Helianthus annuus syncotyleus* he obtained no tricots in ten years, after examination of thousands of seedlings; tricots occasionally occur in *H. annuus variegatus*. Possibly De Vries had here either an example of an oligogene controlling cotyledon manifestation, like the scute condition in *Drosophila* which reduces bristle number, or the strain had been reduced to a homozygous condition.

De Vries (1910) believed that fusion of cotyledons sometimes causes pressure on the plumule, so interfering with growth. Such seedlings grow slower at first compared with normals and he suggested this was why syncots are rarer than tricots. Sometimes the plumules are too weak to split the lower part of the fused cotyledons, while some of the seedlings lack stems, or produce only one or two leaves and then the terminal one stops growing. De Vries (1910) asked whether "the inhibition of the growth may not both here and elsewhere have some other cause also"? There is a relation between syncotyly and upset in leaf arrangement; unequal size of paired leaves; spiral fusion; fasciation; pitcher formation of leaves but these need not always be amongst the syncots of the culture, as in *Coriandrum sativum*.

De Vries (1910) obtained large quantities of syncotyls from his tricotylous race of *Mercurialis annua* but as these had retarded plumule growth he worked with *Helianthus annuus*. As isolated plants set no seed, he was probably using an outcrossing species. He considered that complete fusion was normal, the various stages being only plus or minus variants of this condition. De Vries (1910) gave three interesting selection tables showing:

(a) Selection for hemi-syncots. These gave syncotylous races of average value like selection for true syncots.

(b) Selection of individual syncots which produced the "highest hereditary values". The effects of this selection were noticeable as in each successive generation the proportion of syncots producing larger proportions of syncotylous offspring gradually increased.

(c) Backward selection, or selection in what De Vries called a *minus* direction, by selecting parents in each generation which produced the largest proportion of dicots. The effects of this selection were very marked in increasing the proportion of offspring with large quantities of dicots. Even so after four generations of selection the race still contained individuals capable of giving 65-75 per cent syncots. Therefore re-selection for syncotyly would still be possible.

De Vries (1910) also investigated the effects of environment on tricotyly and syncotyly. He found that the chances of obtaining anomalies increases with the vigour of the seeds which produce them. Manuring with superphosphate compared with hornmeal increased tricotyly frequency in *Oenothera hirtella* and *Helichrysum bracteatum* but soil conditions did not affect syncotyly in *Helianthus*. Increased pleiocotyly goes with diminution of seed yield; this he attributed to unfavourable conditions. Both dicots and tricots of *Antirrhinum majus* give considerable increases in pleiocotyly when cultivated in the shade compared with when they were

grown in the sun. Earliest seeds on a plant do not give any more tricots than later seeds; in *Helianthus annua syncotyleus*, seeds from outer whorls generally have slightly more syncots. Improved conditions in early growth stages have a small influence in a " positive direction "; crowding later on, or partial leaf removal has no effect in *Helianthus*.

Besides the above studies, De Vries (1907) also discussed " The Association of Characters " in his work on *Plant Breeding*. He gave many examples of what are now known to be pleiotropic effects of genes, e.g. the distribution of anthocyanin in various parts of a plant, and of what may be interpreted as linked genes with only occasional crossing over. He referred to the indirect effects on the whole plant by the alteration of part of it. For instance, trees with seedless fruits are less exhausted by fruit-bearing than ordinary varieties. De Vries (1907) also realized the practical possibilities in plant breeding by selecting for pleiocotyly. He stated, " Years ago I was impressed by the fact that deviations in the seedling plants are often followed by abnormalities in the adult state. By this means, the cotyledons of the seedlings may give indications of what is to be expected," and that, " By choosing the stray seedlings with three seed leaves or with Connate Cotyledons . . . the chance of producing monstrosities on a given space being proportionally increased."

Pleiocotyly and Evolution of Angiosperms

As early as 1688 Ray had grouped flowering plants into two groups:

(a) Dicotyledons — " binis cotyledonibus "

(b) Monocotyledons — " singulus aut nullis cotyledonibus "

Besides this primary classification there are a number of other characters belonging to each group. These are summarized in Table 4. As the classification depends also upon other characters which are associated with cotyledon number, the problem is to determine whether these characteristics have occurred as a result of, or parallel with, the selection for one or two cotyledons or both. At present this is impossible to determine on the evidence. Many monocot dicotyledonous seedlings I have examined in my own experiments were normal; some had double stems and had monarch instead of diarch roots (Haskell, 1951). Thus although correlated responses (Wigan & Mather, 1942) are associated with monocotyly, none of the characteristics of Monocotyledons were observed in the selections.

TABLE 4—DIFFERENTIATION WITHIN THE ANGIOSPERMS

Monocotyledons	Dicotyledons
Seeds albuminous (exceptions, e.g. Orchidaceae, and aquatic species).	Seeds exalbuminous (exceptions, e.g. in Ranunculaceae, Umbelliferae).
Primary root replaced by other roots developed from base of stem.	Primary root persists and forms root system of adults.
Normally hypogeal wholly or partially (exception, e.g. onion).	Epigeal (exception, e.g. when cotyledons are very large).
Stem with closed, scattered bundles.	Stem with open bundles usually in ring.
Leaves with parallel veins (exceptions).	Leaves not veined.
Flowers with parts in 3's.	Flowers with parts in 2's, 4's and 5's (rarely in 3's).

Most Coniferales have two cotyledons. Exceptions are *Sequoia* with 4 to 6 cotyledons, and Pinaceae which have several each with a single vascular bundle, the frequency distribution of cotyledon number in the Pinaceae being a good indication of different species, like the distribution of their pollen types. Sargent (1903, 1908) from a study of monocotyledonous seedling anatomy provided evidence on the derivation of monocotyly from the dicot condition. The young monocot epicotyl contains a single ring of collateral bundles, which may even show cambial traces so resembling Dicotyledons. The wide distribution of monocotyly through the Dicotyledons may indicate this is not due to inheritance from a common ancestor

but due to the influence of environment. One characteristic of these "pseudo-monocots" is that they tend to have bulbous underground organs. Sargent states that, of 20 genera having seed leaves fused for some distance upward from the base, the majority have a tuberous hypocotyl. This may have some connection with a previous (semi-) aquatic existence.

Coulter and Land (1914) from investigations of *Agapanthus umbellatum* (Liliaceae) concluded that monocotyly is not the result of two cotyledons fusing, nor the suppression of one. It is the continuation of only one growing point on the cotyledonary ring of the embryo instead of two. Similarly, polycotyly is the appearance and continued development of more than two growing points on the cotyledonous ring. This is true for Cycadophyta, where the two large embryos are free for four-fifths of their length but have bases united into a short tubular sheath enclosing the stem and young leaves.

Buchholz (1919) found many instances of fusion of primordia of cotyledons but none of splitting. Reduction of cotyledon number resulted from their fusion and gave rise to a cotyledonary tube. Thus polycotyly is primitive and dicotyly is derived by either general fusion of many cotyledons into two groups or by an extremely bilabiate development of a cotyledonary tube. On the whole, then, embryological evidence shows that a large number of cotyledons is a primitive character, and the dicotylous and monocotylous states are derived from this.

Another problem pertinent to the phenomenon of pleiocotyly is that concerning the origin of the Monocotyledons. For instance, whether they separated from the Dicotyledons through a Ranunculean group and then diverged, or originated from the proto-angiosperms. Campbell (1940) considered that the division of the Angiosperms into Monocotyledons and Dicotyledons was not entirely natural. This might be expected on Engler's (1926) hypothesis that Angiosperms have originated from "proto-angiosperms" of the Mesozoic; these were an extensive complex of forms. Selection might have led to a single cotyledon in some groups and two in others.

Engler (1926) postulated that a mutation in a single individual of a proto-angiosperm group might have appeared in a similar but not identical form in other individuals. This would perhaps give rise to different races with much in common but which could hardly be traced back to a common ancestral form. If natural selection acted by selecting for monocotyledony then correlated responses would come into play. The correlated responses to selection for pleiocotyly, although unpredictable, fall into certain types such as multiple or lack of branching, fasciation, chlorophyll deficiencies, changes in floral morphology, fertility changes and anatomical differences of roots (Haskell, 1954). As these always seem to be associated with pleiocotyly selection of outbreeding species, and the proto-angiosperms and their derivatives were wind-pollinated, they may be considered to have played a role in effecting the morphological differences between the Monocotyledons and Dicotyledons.

If a pleiocotyly series in, for example, *Brassica* is examined, it is seen that there is with certain exception a gradual reduction in size from dicots to tetracots. This is true not only for individual cotyledon size but also for total area. It is clear that under contemporary conditions the true dicot condition is, morphologically, most beneficial to the seedling. This may, however, be due to physiological balance resulting from selection for dicotyly. If this is so, then we can understand why natural selection has promoted the spread of dicotyly. There has been selection of seedlings with maximum of photosynthetic area and with least mechanical difficulties at germination. For instance, in dicotyledonous species such as Cruciferae one cotyledon is folded over the other when they are in the testa. On germination there is clearly one larger and one smaller cotyledon; this difference becomes less pronounced as the cotyledons expand to their full size. This size difference is a modification due to adjustment to environmental conditions by selection. In the water chestnut (*Trapa natans*) this difference is very marked. There is a larger cotyledon which is liberated and a small scale-like one which is retained inside the

seed coat. In *Streptocarpus*, at first there are two identical cotyledons: after leaving the seed coat, one is retarded and dies, while the other becomes a green foliage leaf. In the specialized dodder (*Cuscuta*) there are no traces of cotyledons; this modification may have been brought about by selection for an unusual life-cycle of parasitism. Thus the environment of the seed at germination has played a role in directing which way selection should act on pleiocotyly.

Although pleiocots less than true dicots are somewhat rare, some of the schizocots are interesting. For instance, in radish they are tubular at the base, which the growing point has to penetrate. Sometimes this is delayed so that the buds are severely damaged before they can emerge. Obviously this places such seedlings at a disadvantage compared with true dicots. Occasionally the cotyledon base is incompletely tubular, with one side exposed forming a groove. The growing point can thus grow up part of the way along the tube and then the bud is able to expand on one side.

If we now imagine the cotyledon blade to be absent one then has a condition very close to that occurring in most Monocotyledons. It is equivalent to the scutellum surrounding the apical bud. *Agapanthus* (Liliaceae) sometimes has two such cotyledons. This would not be unexpected as a wide gamut of pleiocotyly would have existed in the proto-angiosperms and monocotyly is only one end of the range. Monocotyly is still possible for it is fairly common in many dicotyledonous families such as Cruciferae, Umbelliferae and Solanaceae. As it is polygenically controlled (Haskell, 1949) it is possible to conceive that the Monocotyledons are not one group derived by a single major gene mutation from the dicotylous condition, but derived by selection of polygenes controlling cotyledon manifestation. They could thus have arisen quite readily in any of the ancestral groups which gave rise to the Angiosperms. The evidence thus lends support to Engler's hypothesis.

Loss of the cotyledon blade may be due to selection acting on the seeds germinating in muddy conditions. By comparison, it is known that aquatic plants often have much divided, filamentous leaves; this is an adjustment to the fluid medium in which they grow. Further modification has come about due to adjustment of the tubular cotyledon stalk to the embryo-seed relationship. Seward (1941) has pointed out that at one stage in the Cretaceous period when Angiosperms first appeared there was widespread marine transgression; vast regions of dry land were flooded. He asked, "May we not see in this sinking and flooding a possible influence on the course of evolution in the organic world and almost world-wide interference with the physical environment which had its repercussion in the altered trend of plant development?" As already pointed out, monocotyledonous Dicotyledons are especially prevalent among aquatic plants; also a third of the Monocotyledon orders are aquatic compared with 4 per cent in Dicotyledons.

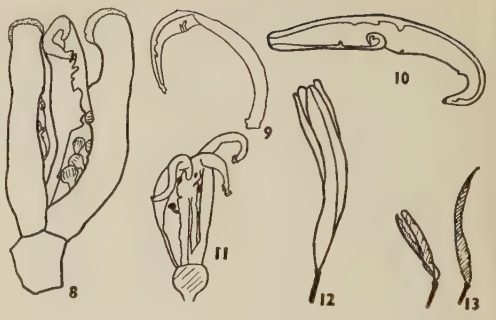
Most of the flowering-plant remains, especially leaves of the Cretaceous, are of the dicotyledonous type, although there is evidence that palms flourished in the latter part of this period. The Pteridosperms and Angiosperms show several features in common. For instance, *Lagenostoma* and *Trigonocarpus* may be compared with seeds and carpels of *Myrica Gale*. Similarly the Pteridosperm *Medullosa* was once described as the stem of *Zea mays* owing to their remarkable similarities. The Cycadeiodea were dicotylous. Thus proto-angiosperms already possessed characteristics of both angiospermous groups. Possibly their separation followed on the differential selection for cotyledon character, as a result of correlated responses or other multiple responses as a result of evolution in different habitats. The Monocotyledons are a group of families which no longer need be considered as derived from a common ancestor. At one time they passed through a special (aquatic) environment and only drastic modification allowed them to survive; then they returned to dry land. Selection during conditions such as these would have brought with it correlated responses in their morphology. Thus the Monocotyledons may be expected to have certain common properties, such as floral parts

with similar numbers in the whorls and lack of cambial growth.

Taking the action of selection even further, it should be possible to predict that Monocotyledons will eventually be found that possess no cotyledons at all, like *Cuscuta*. This possibility cannot be ruled out as there are a number of examples of dicotyledonous species with more or less complete suppression of one of the cotyledons. This leads to conditions intermediate between Dicotyledons and Monocotyledons and comparable with forms like *Dioscoea* and *Zannicellia* with terminal stems and lateral cotyledons. Among these pseudo-monocots may be included *Cyclamen, Abronia, Ranunculus ficaria*. In *Delphinium nudicaule* the two cotyledons are united into a tube with the plumule at the bottom, rather like those of Monocotyledons. In *Podophyllum* (Berberidaceae) there is a similar condition and the first foliage leaf has bilobed lamina closely resembling a pair of united cotyledons.

The opposite theory of Hutchinson (1934) on the origin of the Monocotyledons is that they have descended from a group of flowering plants closely resembling the Ranunculaceae, this being a primitive family which forms the link between Monocotyledons and more specialized Dicotyledons. Two orders of the Diapetalae, i.e. the Centrospermae and Ranales, show resemblance with the Polygonales on the one hand and the Helobiales on the other, and this evidence has been used to support the theory. However, these resemblances may be homoplastic, or both Ranales and Helobiales have originated independently from similar proto-angiospermous ancestors. Evidence on this question might be obtained by selecting monocot seedlings of the lesser celandine (*Ranunculus ficaria*) to see whether they would show monocotyledonous morphology after a few generations of selection.

If, as Gager (1920) thought, the appearance of supernumerary cotyledons amongst Dicotyledons was a reversion to a more primitive condition, possibly then they should show more primitive characters. Does the evidence from correlated responses support this theory?



FIGS. 8-11 — Flowers from tricot Kohl rabi (*Brassica oleracea*). Fig. 8. Sepals removed. Note absence of anthers, distributed stylar tissue and naked ovules. Fig. 9. A flower represented by bract-like structure with stigmatic surfaces. Fig. 10. Bract with stigmatic tip covering a flower. Fig. 11. Flower, facing inwards; anthers, petals and calyx are absent. Note brown ovules, withering stigmas and central placenta.

FIGS. 12, 13. Pods from a tetracot strain of "Harbinger" Cabbage. Fig. 12. Polylocular dried pod. Fig. 13. Septa. Left, twinned: right, normal.

The abnormal flowers of the tricot Kohl rabi, illustrated in Figs. 8-11, indicate that selection for pleiocotyly brought with it correlated morphological changes of a primitive type. Here naked seeds on incompletely closed carpels bear a strong resemblance to the condition of Reseda, belonging to the closely related family Resedaceae. This indicates that features of two closely related families may be bridged by these changes. Similarly, in pleiocotylous turnip, vivipary is a primitive condition.

Selection for pleiocotyly, therefore, offers a new technique for the plant taxonomist. He may now be able to produce in greater quantity teratological forms (cf. Figs. 12 and 13) recognized as being of value in the interpretation of taxonomic problems of normal forms. For instance, the Kohl rabi (Figs. 8-11) gives evidence on the origin of the stamens and carpels, i.e. whether these are metamorphosed foliage leaves or organs *sui generis*, and supports the former view. Similarly Holtorp (unpublished) has obtained interesting abnormal flowers on *Antirrhinum* and *Cheiranthus* of morphological interest. Obtaining further floral abnormalities may help to elucidate some of the work of Miss Saunders and H. Hamshaw Thomas on carpel morphology.

Summary

Pleiocotyly, the variation in cotyledon number, is most commonly seen in Gymnosperms and Dicotyledons. Polycotyly is more primitive than monocotyly. Pleiocotyly is reviewed historically in relation to morphology, anatomy and physiology. Environmental effects and its genetical basis are recognized.

De Vries' experiments on selection for pleiocotyly are re-evaluated in the light of modern theories. The difference between inbreeding and outbreeding species is emphasized and the bearing of pleiocotyly on the separation of Dicotyledons and Monocotyledons is considered. Monocotyly may have been favoured during adaptation to a previous semi-aquatic existence.

Correlated responses affecting morphological characters of the adult may have accompanied natural selection for monocotyly acting during early stages of germination. Hence Monocotyledons need not necessarily be derived from a common ancestor.

Pleiocotyly selection offers plant taxonomists a new technique. There is increased opportunity of producing teratological forms, perhaps with characters bridging different families.

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THE DURIAN THEORY EXTENDED — II. THE ARILLATE FRUIT AND THE COMPOUND LEAF

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The Arillate Follicle Large, Parenchymatous and Spiny

That this was the precursor of modern angiosperm fruits has been forced upon me by the study of tropical plants. The mechanism was simple, histologically unspecialized, and sufficient, no doubt, for witless creatures. It survives no longer in its entirety, because the forest and the mind have evolved. Thus, the arillate follicles of Annonaceae, Myristicaceae, Connaraceae, Leguminosae, and Sterculiaceae tend to be lignified, spineless, and few-seeded. Those of Dilleniaceae are smooth, small-seeded, limited within the sepals, and tending to be capsular. The spiny arillate capsules of *Durio* and *Sloanea* are heavily lignified and tending to indehiscence, while those of Celastraceae and Sapindaceae are few-seeded and tending to small fibrous capsules. The scitamineous capsule is far derivative as an inferior ovary. The spiny fruits of *Victoria* and *Euryale* are green, thin-walled, and with vestigial arils. The nearest approach to the primitive state is the fruit of some species of *Tabernaemontana* (Apocynaceae), with fleshy orange follicles covered with fleshy processes and filled with many, moderately large, arillate seeds. The Apocynaceae are advanced in most other respects, and this point shows how impossible it is to estimate the general level of evolution of a family. If palaeobotany tells anything about flowering plants, it is that modern genera existed in the early Cretaceous, from which one may infer, with Croizat (1952), that the origin of their families must have been in the Jurassic or earlier. The significance of *Tabernaemontana* cannot be realized except by hypothesis covering the evolution of the angiosperm forest. This was the scope of the durian theory, wherein I endeavoured to show how much information lay in the tropical forest. An obvious conclusion, which I did not immediately grasp, is that the majority of angiosperm families must have evolved in the arillate, megaphyllous, pachycaul stage before angiosperm forest existed. This long view needs emphasis, because there is too facile a tendency to interpret existing genera and families on their subsequent polyphyletic tendencies. The problem of *Tabernaemontana* is one of Jurassic vegetation, and not whether

Apocynum is more advanced than *Ranunculus*.

SIZE — It is difficult to say how large the primitive arillate follicle may have been. I do not doubt that it may be conjectured with some accuracy when more is known of tropical fruits. To judge from *Delonix* (Caesalpiniaceae), it would have contained about 50 plump seeds 1 cm. long. The greater number, up to 120, in some species of *Cassia* may have arisen from basipetal extension of the phyllodic carpel, coupled with decrease in seed-size, just as one finds *in extremis* among the orchids. But the modern legume is functionally terminal and may be an enlargement on the lateral follicle as seen in *Archidendron* (Mimosaceae), *Xylopia* (Annonaceae) and *Sterculia*, with 10-30 seeds. Evolution has been mainly towards fewer and larger seeds, or more and smaller seeds. We lack, of course, generic evaluation.

SUCCULENCE — That the cortical tissue of the primitive arillate follicle was parenchymatous and fleshy is what can be observed in existing fruits (*Paeonia*, *Tabernaemontana*, Connaraceae, and so on). The conclusion follows from the consideration that lignified and desiccating mechanisms are histologically and physiologically specialized. Hence I assume that the dry, lignified or fibrous follicle or capsule and the drupe are derivative. Parkin (1953) enters a *caveat* that, in some cases, the berry may have evolved from the dry capsule. Ridley (1930) argued both ways. I have seen no convincing evidence for Parkin's point of view in tropical plants, though I am unable to answer the special problems which he gives. I certainly disagree with Ridley's examples in which he derives the large fleshy capsule of *Fagraea* sub-genus. *Eufagraea* (Loganiaceae) and that of *Momordica* (Cucurbitaceae) from berries. He argued that they were adaptations to permit seed-dispersal, the first from the small berries of the genus, which animals can swallow, and the second from the large cucurbitaceous type of berry which animals can tear open. I fancy that all this evolution took place before there were modern birds and mammals, and there is no reason to doubt that the normal evolution from dehiscent to indehiscent fruits took place also in their genera. *Fagraea*, with large terminal flower and stout twig, shows the more pachycaul state of the family, while the small-berried small-flowered, small-leafed, and slender-twigged *Cyrtophyllum*, from which he would derive *Fagraea*, is the advanced state. With regard to *Hypericum*, mentioned by Parkin (1953), there is a parallel in the same family between the berries of *Vismia* and the dry capsules of *Cratoxylon*. They may be independent baccate and capsular derivatives of the arillate fruit, as one sees more readily in the allied Guttiferae, all referrable to Jurassic evolution. The same divergence may have occurred as early in *Hypericum*. There is a similar problem with *Paris*, *Dianella* (Liliaceae, as described by Ridley, 1930), *Cucubalus* (Caryophyllaceae) and *Crambe* (Cruciferae). Equally puzzling is *Prunus amygdalus*, the leathery exocarp of which dehisces, suggesting a more primitive state than the succulent plum and cherry, even than the five-carpelled *Nuttallia*. These are problems that, one hopes, will draw carpology from its dormancy in museum cabinets. Nevertheless, one must consider the likelihood of a long ancestry for genera, as well as for families, with much extinction of progenitors, particularly in extra-tropical plants. Thus, the tropical arborescent Cordioideae with berries appear antecedent in this respect to the extra-tropic boraginaceous herbs; similarly with the Verbenaceae and Labiatae, or the Araliaceae and Umbelliferae. The berry of *Actinidium* seems directly referrable to the exarillate capsule of *Dillenia*, just as the berry of *Garcinia* is to the fleshy and arillate capsule of *Clusia* (Guttiferae).

In the case of Monocotyledons, I disagree also with Parkin's (1953) *caveat*. The banana is surely a fleshy sort of berry derived from the arillate scitamineous capsule. In the Helobieae, not only has *Posidonia* a fleshy fruit, but that of *Enhalus*, which is the marine counterpart of *Vallisneria*, is fleshy, spiny and large-seeded, as the durian theory postulates, though both appear to be exarillate. Florally and vegetatively these genera are as advanced as *Tabernaemontana*, but that

is no proof that their fruits are without Jurassic traces. Indeed, these genera have much the same interesting aquatic status as Nymphaeaceae, to which I will refer. With regard to the large succulent red drupes of *Melocanna* (Bambuseae), I find no difficulty in comparing it with the baccate, one-seeded fruits of *Dracaena* and the palms. The caryopsis is a structure as simplified, specialized and derivative as the haulm, leaf, inflorescence and floret of the grass: it presupposes a nut (Dendrocalamus), which may well be derived from a fleshy fruit, as in the palms. We meet in the Gramineae the same problem as with the arillate fruit in most families; that is a rare form of fleshy, animal-distributed fruit, and a common form of dry fruit fit for any manner of dispersal; orthodoxy has the rare fruit as advanced and the common as primitive. But all this happened millenia ago, at the beginning of the modern vegetation.

Spines — Many arillate fruits or, according to the durian theory, their immediate derivatives have spines. They occur, too, on advanced fruits, as boraginaceous nutlets, Ranunculus achenes, umbelliferous mericarps, cruciferous and papaveraceous capsules, and even orchidaceous. Modern power may render vegetable weapons ludicrous, but anyone who has traversed tropical forest will know what it is to kick against the pricks. The association of epidermal outgrowths, whether spines, scales, or glands, with fruits needs enquiry, particularly when they are connected with peltate scales (*Durio*) and stellate hairs (*Mallotus*), or with glandular hairs as in *Dyctamnus* (Rutaceae). I interpret these enations as relics from primitive flowering plants, which have come to have minor importance in modern vegetation. *Lyginopteris*, I would recall, was reconstructed mainly through its stalked glands (Scott, 1924). Were the primitive flowering plants glabrous? Have the simple, stellate, peltate and glandular hairs, as well as other enations, evolved polyphyletically in modern families and genera from a uniform field of epidermal cells controlled for non-excrescence? Or are the glabrous plants derivatives? I maintain the second view, because there is so much evidence for the simplification and loss of hairs in modern stream-lined plants, with their hairy and glabrous forms. The problem to be faced is the nature and extent of the enations of the primitive plants, and for this one must enquire into their occurrence on living plants. The dissection of living growing points will soon convince one that the glabrous state is the derivative.

Many forest palms bristle with extraordinary enations, protecting their soft and edible tissues. Other palms are protected by massive, fibrous leaf-sheaths, or by chemical unpalatability; the enations are then vestigial, often serving as lubricants in the bud, and then lost as deciduous tomentum, unappreciated by the botanist, but valued in the forest as tinder for the spark. The enations vary from simple or stellate hairs to extensive epidermal outgrowths building spines and combs of spines. In the lepidocaryoid palms the enations on the ovary are set in whorls and develop into an armour of backwardly imbricating spines on the fruit. As this ripens, the tissue between the scales softens and the scales flake off the edible mesocarp, or the tissue dries up and the scales can be detached in a pellicle, as monkeys know. The basipetal growth of the ovary, both before and after fertilization, causes the backward deflection of the spines by pressing their tips against the rigid perianth. This growth of the enlarging palm-fruit frequently exposes the soft young tissue surface toward the base, and this, as entomologists know, is the "Achilles' heel" for sucking insects. The scales, therefore, function both against insects and against larger creatures that would eat the unripe fruit. They function as do the enations on the leaves and stems, and there is no reason to dismiss them as modern decoration. On the durian theory, they are at once intelligible, and it comes then as no surprise to find tubercles on the fruits of other diversified genera of palms, as *Teysmannia*, *Pholidocarpus* and *Phytelephas*. Palms are usually regarded as a liliaceous product, but the multistaminate and apocarpous condition of many is more primitive than any liliaceous. Their fruits,

which are drupes or nuts, imply antecedent follicles or capsules, which the durian theory invests with spines and arils, very much as the scitamineous ancestor, and less like the Liliaceae. Such must have been the pre-Cretaceous palms. Cyclanthaceae fit the palm-series as herbaceous derivatives, comparable with gingers and arrowroots.

Another family in need of attention because of its many primitive marks is the Nymphaeaceae. *Victoria* and *Euryale* are aquatic plants abounding in spines, and it is difficult to see how the polyphyletic evolution of spines can be maintained both for palms and submerged water-lilies. Spines may well supply the key to the history of the epidermis, which cannot have been uneventful.

In searching for evolutionary and morphological clues, I have found the occurrence of spines most useful. The dry, spiny capsules of *Datura* connect with the very spiny, thick stems and pinnate leaves of the least specialized, tropical species of *Solanum*. The baccate fruit of *Solanum* would appear obviously to have lost its armour, but a spiny, capsular and arillate pachycaul plant with pinnate leaves is the equally obvious conjecture for the solanaceous ancestor. The dry spiny capsule of *Allamanda* (Apocynaceae) and the softly spiny, berry-like fruit of *Daturicarpa* (Stapf, 1921) connect with the arillate follicles of *Tabernaemontana* and indicate a similar pachycaul ancestor for the Apocynaceae, which is why I wonder at that curious relic *Pachypodium*. *Momordica* is the one genus of Cucurbitaceae with fleshy, dehiscent fruits. They are pseudo-arillate, the function of the aril having been transferred in this family apparently to the pocket of endocarp in which the seed develops, and they show all stages in the degeneration of the spines from bristles in *M. foetida* to the slight humps on some indehiscent varieties of *M. charantia*. The spines disappear as the berry perfects. They persist, nevertheless, in other genera such as *Ecballium* and *Sechium*, and perhaps, too, as the warts of *Cucumis*. A fleshy spiny arillate capsule is the obvious conjecture for the primitive cucurbitaceous fruit, as it is for the fruit of *Carica papaya*, which in its pachycaul habit and cucurbitaceous affinity may display the ancestral form of the gourds. In the Flacourtiaceae, the arillate fruits of *Carpotroche*, *Meyna* and *Oncoba* are variously ornamented with excrescences, while the exarillate *Buchnerodendron* has a spiny fruit. *Bixa*, with vestigial aril and red sarcotesta, has a dry bristly capsule. The large and predominantly arillate tribe Nephelieae in the Sapindaceae has many spinous and tuberculate fruits, and this family, which is still so abundant, must surely illuminate the vestigial Hippocastanaceae with smooth arillate fruit (*Billia*) and spiny exarillate fruit (*Aesculus*). Comparative systematics gives understanding. *Sloanea* and *Durio* show a parallel in their families from the spiny arillate capsule to the spineless, indehiscent and exarillate derivatives in other genera. Similarly with the Sterculiaceae and Euphorbiaceae there are traces of the spiny fruit. The arillate *Commersonia* (Sterculiaceae) has woolly spines, as do the capsules of *Buettneria* and *Guazuma*, which may indeed have vestigial arils. *Theobroma* has traces of stout spines similar to those of *Momordica*. No arils are known for the Tiliaceae, but several genera related with *Apeiba* have indehiscent, spinous or muriculate fruits strongly resembling rather small, indehiscent durians: they, too, may have vestigial arils, and it is the value of the theory that it can direct attention to such objects of botany. Then, again, several of the Turneraceae, with small arillate fruits, have a minutely spinous exocarp, just as in the Cannaceae. The peculiarity of *Flindersia*, now transferred from the Meliaceae with many arillate fruits to the Rutaceae which are almost entirely exarillate, is emphasized by its muriculate fruits. How interesting, then, is the presence of spinous capsules in two subgenera of the arillate *Euonymus*, though Blakelock (1951) in his monograph took the orthodox attitude that " a round smooth capsule is more likely to be primitive than one deeply lobed or with outgrowths ". Why, then, have a few species in *Euonymus* produced this new evolution ? Is it decoration ? The answer is revealed in the fact that the smooth-fruited

E. europeus is poisonous in all parts except the aril. As with the palms, mechanical protection gives place to chemical, and the enations remain as vestigial decoration. It is customary to read also, I believe, the evolution of thorny pedaliaceous fruits from the spineless to the spiny condition, without advancing botanical evidence (Hutchinson, 1946); the reverse is more likely to be true.

The presence of enations on fruits is a valuable, if curious, guide to research. That is why I regard the rare occurrence of spiny fruits in the Leguminosae as important. They occur in *Mimosa* and its ally *Schrankia*, in *Sindora* (Caesalpiniaceae), and in the papilionaceous *Pterocarpus*, *Glycyrrhiza*, and *Medicago*. All are modern genera, but they may stem from the Jurassic and so they may carry traces of the now almost extinct spiny arillate legume. The spines of *Glycyrrhiza* and *Medicago* have vascular bundles, as they have in Bombacaceae and Euphorbiaceae, in which families they may be connected with the cortical vascular bundles. Spines with vascular bundles also develop on the fruit of *Dyctamnus*, though they must be vestigial for they do not connect with the vascular supply of the ovary; in fact, some of the spines have only one or two tracheids. Have we here a clue to the former complexity of the epidermis ? I cannot believe from my experience of plants that the smooth epidermis, the uniform cortex and the simple ring of vascular bundles, so typical of temperate dicotyledons, make a primitive condition; the very efficiency, lightly sprung with collenchyma, marks derivation. When plants came on to land, animals began to crawl over them. Take a look at an aphid on a moss-rose ! And what climbs a bamboo ? A professor once advised me that Protista were the link between botany and zoology, rather it is the tropical forest, where every subject joins the sister sciences.

The Winged Seed

In *Sterculia* and its ally, *Pterygota*, there is a transition from the fleshy arillate follicle through the slightly lignified, exarillate follicle with fibrous endocarp to the dry, rather woody, though brightly coloured, follicle of *Pterygota*, which is packed with large winged seeds. Loss of the aril has been accompanied by lignification of the fruit-wall, securing dehiscence by the drying out of crossed fibres (? hypodermal fibres of the carpellary leaf-base), rather than by swelling of the tissues, and by multiplication of the ovules which, on enlarging, become compressed and extended at the free end into wings. In the capsular Meliaceae, the difference is tribal, separating the Cedreloideae and Swietenioideae from the mainly arillate and fleshy fruited Melioideae. In the Apocynaceae, the characteristically winged seeds appear related with the arillate seeds of *Tabernaemontana* and *Neokeithia*. Other arillate families, such as Bombacaceae, Guttiferae, Celastraceae, and Theaceae, provide numerous examples, which convince me that all winged seeds are *prima facie* indications of arillate ancestry. Many families lacking arils are thus gathered into the fold, and the winged seed shows how they should be assessed. Thus, the Proteaceae have many winged seeds; *Flindersia*, the rutaceous genus with spinous fruits, has winged seeds ; Bignoniaceae have typically winged seeds, as the Apocynaceae, and some have spinous-muriculate capsules, and so on, with Oleaceae, Rubiaceae, Liliaceae, and Iridaceae. *Iris*, has, indeed, arillate seeds, seeds with sarcotesta, and winged seeds, revealing the derivations of the arillate seed within the limits of a genus, most species having the derivative, unspecialized, and secondarily simple seed. *Xanthorrhoea*, the arborescent liliaceous plant of Australia, has a primitive pachycaul habit and winged seeds, which emphasize its peculiar nature. Even the winged seeds of *Casuarina*, *Nepenthes* and Orchidaceae acquire a new significance. Thus, like the spinous fruit, the winged seed shows where carpological and phyletic research may be profitable. I would apply the same argument to the coniferous seed, the dry cone and winged seed being derived from fleshy structures persistent, if highly modified, in *Taxus*, *Podocarpus*, and *Phyllocladus*, as in *Cycas*, *Ginkgo* and *Gnetum*.

No winged seeds occur, I believe, in the Leguminosae. Their enlarging carpel

compensates ovular growth, and it has, in most cases, suffered reduction in number of the ovules. The winged analogue is, as one would expect, the leguminous samara.

The Dicotyledonous Leaf

Parkin (1953) introduces *Acer* and *Rubus* to show that the primitive dicotyledonous leaf-form may have been the simple lamina. I concluded that it was the acropetally developed pinnate leaf. Troll (1935), in an important paper which seems to have been little studied, concluded that it was the basipetally developed pinnate leaf. Now leaf-shapes can be arranged in all manner of series from cotyledons or bud-scales to highly compound forms, which may lead again to bracts and sepals. The problem is to discover "time's arrow", or the evolutionary direction, which is very different from the transformations that occur on a living plant. I do not agree that the solution, if any, must be fossilized. I doubt if the rocks hold more than a minute fraction of any extinct series of organisms, and then the series require continuous deposition over millennia, if it is to be absolutely indicative. Instead, let us examine the enormous generic and specific diversity among living plants. No fossil record has told us that unicellular organisms preceded multicellular, or isogamy preceded heterogamy. Cannot a principle of evolution be discovered among the thousands of living leaf-forms?

Sapindales — *Acer* has simple, palmate and pinnate leaves. *Dipteronia*, with less evolved schizocarps, represented as a tertiary relic in China, has pinnate leaves. The Aceraceae, as the Hippocastanaceae, are part of the sapindalean problem, and most Sapindaceae have pinnate leaves. But, on one side of the apparently terminal leaflet, there is a small spike, representing the tip of the rachis. This spike needs examination.

My studies on the development of sapindaceous leaves in Malaya have shown me that, as in most cases of acropetal pinnate leaves, which have been so clearly studied by Troll, the construction resembles a dorsiventral branching system with apical growth of the axis or rachis and of the branches or pinnae, followed by intercalary extension to form the leaflets. The pinnae develop acropetally and, usually in the family, alternately. The longer the primordial growth of the rachis the more numerous will be the pinnae. Thus, there may be as many as twenty pinnae on each side of the rachis, as in sapling leaves of *Pometia*, or only one as in species of *Xerospermum*, but the apex of the rachis persists untransformed as the spike. In the case of the bud-scales and the bracts, apical growth stops very early and the base of the primordium enlarges into the scale. There is, thus, a series dependent on the limitation of apical growth. Limitation means specialization. Just as the limitation of the floral axis reduces the magnolian type of flower to the epigynous, or limitation of the apical growth of the inflorescence axis reduces the raceme to the capitulum, so it reduces the pinnate leaf with many leaflets to that with one or none, which is the scale-leaf comparable with the sepal. In some Sapindaceae, however, the spike at the end of the rachis becomes a truly terminal leaflet, exactly as leaflets normally terminate the primary branches of the rachis. Limitation of such a leaf produces the trifoliate, as in many other Sapindaceae, and ultimately the unifoliate as in *Dodonaea*. Thus, limitation of apical growth produces the precise structure, which is the unit-lamina or simple leaf: it is derivative and not primitive. In this light, therefore, I view the pinnate form in *Acer* and *Rubus* as the less evolved.

Unifoliate leaves — The derivation of the simple leaf from the pinnate is shown clearly in the Leguminosae, Oxalidaceae Burseraceae, Rutaceae and Araliaceae, because their leaflets are articulate to the rachis. The simple leaves of *Swartzia* and *Desmodium* in Leguminosae, *Connaropsis* (Oxalidaceae), *Dacryopsis* (Burseraceae), *Citrus* (Rutaceae) and *Nothopanax* (Araliaceae) are articulate to the top of the rachis, which shows that they are terminal leaflets, not webbed pinnate leaves. Other species of their genera, or of allied genera in the case of *Citrus*, show the steps leading through the trifoliate leaf from the imparipinnate. Unifoliate leaves can also be traced by comparative

morphology in genera of Meliaceae, Simarubiaceae and Bignoniaceae. If, nevertheless, one persists in maintaining that structures of limited growth may evolve the means of unlimited apical growth, then one is faced with the conclusion that in these modern instances the family characters of the compound pinnate leaf are being evolved polyphyletically among the present-day species of certain, otherwise specialized, genera. *Swartzia* and *Desmodium*, for instance, would have among their species proto-leguminous leaves, *Citrus* proto-rutaceous, and so on, which is absurd. Not merely is such a conclusion comparable to the derivation of racemes from umbels, or hypogynous from epigynous flowers, but it avoids the problem how the complex organization of the pinnate leaf evolved. What these cases show, as clearly as the capitulum and the inferior ovary, is the specialization of the family character of the pinnate leaf to the unifoliate state of limited growth. Similar argument shows that the unijugate leaf of many caesalpinioid genera is a limiting term of the reduction of their paripinnate leaves, as in *Cassia*, *Brachystegia*, *Heterostemon*, *Copaifera* and *Cynometra*.

Unlimited leaves — The argument places the leaf-form with most limited apical growth as the most derivative. Is there any example of a leaf with unlimited apical growth? Now, as shown by Sinia (1938), and as I have myself verified, certain tropical trees of the Meliaceae have perennial pinnate leaves, the rachis of which ends in a circinate bud covered by incipient leaflets, which it continues to produce for several months or *years*. The leaflets open one at a time, or in pairs, and the rachis extends between the leaflets, carrying the circinate tip further away from the stem. The proximal part of the rachis thickens and develops bark, and its resemblance to a twig is heightened by the shedding of old leaflets as the new ones develop. This shedding of old and renewal of new leaflets may, indeed, be seasonal. Such leaves occur in some species of the genera *Aglaia*, *Chisocheton* and *Guarea*, of which *Chisocheton* is arillate, while the others have a sarcotesta as a derivative aril. They are not absolutely unlimited, because they reach finally a specific length, which may be only some 30 cm. in *Guarea*, but as much as 6 m. in some Malayan species of *Aglaia*, in which cases the leaves function as branches on the straight trunks, which may reach 20 m. high, though the terminal inflorescence imposes some true branching. When the growth of such leaves stops, the whole rachis is shed by abscission from the trunk, exactly as the phyllomorphic branches of annonaceous, myristicaceous and other monopodial trees. Now, *Aglaia* the interest of which I have already emphasized as an arillate genus (Corner, 1953), has also normal pinnate leaves and unifoliate leaves in other species. I can see no escape from the conclusion that such prolonged apical growth, however unsatisfactory, was the nature of the primitive Meliaceous and Sapindaceous leaf: it was closed by the development of the terminal leaflet or, as in most Sapindaceae and Meliaceae, by the arrest of the tip into the abortive terminal spike. Eventually, small and more manageable leaves were evolved, fitting branches that could persist. The rarity of this unlimited pinnate leaf in the tropical forest shows that it has been largely superseded.

Doubly pinnate leaves — In the Mimosaceae the doubly unijugate leaf, which has a petiole and two petiolules each with a pair of leaflets, is clearly the limiting term in reduction of the doubly pinnate leaf. It is seen in *Calliandra* and *Pithecellobium*, with all transitions to multijugate leaves in different species. The doubly pinnate leaf may also simplify to the once pinnate just below the inflorescence. Thus, the very large genus *Inga*, distinguished in the family by its simply pinnate leaves, shows the derived condition with less branching of the rachis. In the Caesalpiniaceae, simply pinnate and doubly pinnate leaves occur among the species of *Caesalpinia*, *Haematoxylon*, *Gleditschia* and *Moldenhauera*, belonging to the Eucaesalpinieae, and even on the same tree in the last two genera, as Troll has investigated for *Gleditschia*. In the Dimorphandreae, with doubly pinnate leaves, *Mora* has simply pinnate and *Sympetalandra*, though described from an inflorescence and a leaf reduced to the simply pinnate state, has

both doubly and simply pinnate leaves. Now, if the doubly pinnate condition is supposed to have been evolved from the simply pinnate, then these living genera show the polyphyletic evolution of the complicated mimosoid, eucaesalpinioid and dimorphandroid leaf, even on the same tree, which I consider to be absurd. The evidence from the comparative morphology of the living species shows the reduction to the simpler state.

In the Leguminosae, the Mimosaceae with primitive floral characters have predominantly the primitive doubly pinnate leaf. So, too, have the Eucaesalpinieae, as the least specialized tribe of the Caesalpiniaceae, while the truly advanced groups both in floral and vegetative form (with applanate foliage), as the Amherstieae, Bauhinieae, Cassieae, Cynometreae, Swartzieae and all Papilionaceae, have the simply pinnate leaf and its derivatives. What of the Dimorphandreae ? They are generally classified as caesalpinioid, but re-estimation will surely ally them with the Parkieae, among the Mimosaceae, as revealed by the close resemblance between *Brandzeia* and *Pentaclethra*. I conclude that the primitive leguminous leaf, though of limited growth, was doubly pinnate. The same must hold for the Sapindaceae.

Highly pinnate leaves — Though I have not examined the families in detail, I think that the three- and four-times pinnate leaf of the Araliaceae, Umbelliferae (see Troll, 1935) and Bignoniaceae will prove to be the least limited and most primitive state in them. Where, then, does the degree of pinnation stop ? That, I feel sure, was the proto-angiosperm problem. It borders on the origin of the leaf as a whole; and the primitive leaf certainly does not exist today.

Webbed leaves — Another modification is found in *Artocarpus* (Moraceae). While *A. anisophyllus* alone has imparipinnate leaves, many species have simple and entire leaves, but they are not unifoliate. Several species, as *A. incisus*, have pinnatifid leaves, in which the main lateral veins end in lobes which correspond with the pinnae of *A. anisophyllus*. In some species there is a transition from deeply pinnatifid leaves, lobed even to the fifth degree in *A. elasticus*, to the simple entire adult leaf with the primary veins incurving toward the apex of the leaf before reaching the margin. These transitions show that the simple leaf of *Artocarpus* is a whole imparipinnate leaf webbed up through reduction of the lobes of the rachis and intercalary expansion of the lamina. Just as the apical growth of the pinnate leaf determines the number of pinnae, so in the webbed leaf it determines the number of main lateral veins. Thus, the webbed leaf can be seen to be limited down to the state with few main lateral veins, exactly as the pinnate leaf. Similar transitions from pinnate to webbed pinnate leaves occur in the Rosaceae, Proteaceae, Geraniaceae, Berberidaceae, Dilleniaceae (as in *Acrotrema*), Fagaceae and Quiinaceae (sec Foster, 1951). The lobed form of *Liriodendron* and *Sassafras* indicates a similar explanation for the simple leaf of Magnoliaceae and Lauraceae. In *Dillenia* the apical growth may reduce from forming some eighty main lateral veins in sapling leaves of *D. reticulata* (about 1·5 m. long) to merely four or five veins in the small leaf of *D. pulchella*. In the Botanic Garden at Rio de Janeiro there is a variety of the rubiaceous tree *Genipa americana* with pinnately lobed leaves, which supplies the only evidence that I know in explanation of the rubiaceous simple leaf.

Palmate leaves — In the alliance of Bombacaceae, Malvaceae, Sterculiaceae, Tiliaceae, Elaeocarpaceae and Euphorbiaceae, the pinnate leaf does not occur. Their leaves are palmate, trifoliate or simple. Most species of *Tarrietia* (Sterculiaceae) have 5-11 separate leaflets, but *T. simplicifolia* has a single blade geniculate to the petiole, as are the individual leaflets in the other species. This blade is the median leaflet and shows that the simple leaf is here a unifoliate palmate leaf. Probably this is the explanation of the simple leaves of *Durio*, *Coelostegia* and many other Bombacaceae. In *Sterculia* a few species have palmate leaves, but more have palmately lobed leaves which indicate a webbing of the leaflets, and others have simple cordate leaves, which are wholly webbed palmate leaves. *Pterospermum* (Bombacaceae), *Hibiscus* (Malvaceae), *Jatropha* (Euphorbiaceae) and

Triumfetta (Tiliaceae) show the same degrees of webbing. So does *Ficus* in contrast with *Artocarpus*, and also *Vitex* (Verbenaceae). These genera show, in consequence, remarkably similar leaf-forms.

Here, then, we have the intrageneric derivation of simple leaves from palmate leaves, resulting in the unifoliate and the webbed palmate. Likewise the trifoliate leaves of these families are trifoliate palmate leaves, not trifoliate pinnate leaves. I argue, again, that it is absurd to suppose that these genera are evolving the elaborate palmate leaf among their living species. What they show is the parallel limitation of apical growth of the palmate leaf, accompanied by webbing. In this category belong the palmate, palmately lobed and simple leaves of *Acer* and *Rubus*: they are, as Troll has shown, basipetal leaves.

Basipetal pinnate leaves — Acropetal leaves show their mode of development both by acropetal growth of the primordium and by acropetal expansion with concomitant colouration and hardening. Many dicotyledonous leaves, however, after a brief period of apical growth, then develop basipetally. Thistles are, perhaps, the best example, for the spines are the tips of the leaves basipetally hardened, while the soft stalks, protected in the spiny rosette, are the basipetally growing regions which intercalate new pinnae. In general, basipetally developed pinnate leaves have the pinnae diminishing in size down the rachis, whereas they diminish in size up the rachis in acropetal leaves, though there are exceptions, as Troll has shown. Most, if not all, Compositae, Valerianaceae, Polemoniaceae, Solanaceae and Cruciferae have these basipetal leaves. They may become webbed into a simple basipetal lamina, as in *Lactuca*, or reduced to the terminal pinna, as in *Brassica*, both examples being employed in cultivation for the bunched leaves resulting from the manner of growth and difficult unfolding. Further, the basipetal leaf reduces to a bract, which may be a leaf-tip as in thistles, or a leaf-base as in sunflowers. I suspect the phyllaries of the capitulum to be as diverse.

Yet another example of basipetal leaves occurs in the longitudinally veined leaves of *Plantago*, *Gentiana*, *Cinnamomum*, *Bupleurum*, *Eryngium*, *Ranunculus*, and many Caryophyllaceae and Melastomataceae. Basipetal growth extends enormously the part of the lamina between the first, or basal, lateral veins and the second pair, while the distal and acropetally developed part of the lamina scarcely enlarges. Thus, the first primary veins, which are the main basal veins, are drawn out parallel with the midrib, to give the phyllodic leaf. In *Piper* and many Melastomataceae the subsidiary, or basipetally intercalated, basal veins may be incorporated to give the 5, 7, 9, or 11 longitudinal veins. Striking examples of the phyllodic leaf occur, as one would expect, in families with basipetal pinnate leaves such as Compositae (*Tragopogon*, *Scorzonera*), Ranunculaceae, and Rosaceae (*Cliffortia*). *Lathyrus* (Papilionaceae) has this character in its leaflets, in contrast with *Vicia*, and the *Lathyrus*-leaf, as is well known, reduces to the phyllodic and merely stipulate states. Aphyllous winged stems are also basipetal effects, as can be traced in *Centaurea* and *Cirsium*.

Plantago is most instructive. It shows the transition from pinnate to palmate leaves and from these to the phyllodic. Some species have phyllodic leaves with pinnate vestiges, as teeth. This and many other examples will be found in Troll's useful paper, where he shows how the palmate leaf is a basipetally developed pinnate leaf without extension of the rachis between the leaflets.

Regarding the Melastomataceae, I think that the Memecyleae, with normal pinnate venation, will prove the key to the phyllodic form of leaf which is otherwise typical of the family. There is a tendency to exclude the tribe without this being appreciated.

Family variation — Some families have a fairly constant leaf-form, but in others it is most varied. The Araliaceae have pinnate and palmate leaves, each reducing to the simple unifoliate or webbed form. Thus, the palmate series of reduction to unifoliate leaves can be read among the species of the large genus *Schefflera*, beginning with forms that are palmate to the fourth degree. *Nothopanax* shows the pinnate series. *Hedera*, however, has a

webbed palmate leaf, like so many tropical members of the family. In contrast with this tropical variation, the Rosaceae show as great a range of leaf among temperate forms. Parkin (1953) suggested that climate may have been effective in the evolution of leaf-form, but I can see little evidence beyond a selective action. In the Malayan rain-forest the large euphorbiaceous tree *Emblica* has a very specialized form with tiny leaves arranged in phyllomorphic twigs and with merely scale-leaves on the main stems, but it grows with the leguminous *Parkia* which has doubly pinnate leaves with as tiny leaflets and both grow with *Artocarpus*, which has large and small, simple or pinnate leaves, and they may all be overgrown by *Bauhinia*, which has its own very peculiar leaf (van der Pijl, 1952). There is no evidence that any of these genera evolved outside of the tropical rain-forest. So one must conclude that the leaf has evolved as part of the shoot-mechanism, in which progressive simplification has occurred in many ways to render the branch-systems more efficient in their elevation of the forest-canopy.

Conclusion — The simple elliptic leaf is a polyphyletic confusion. Its nature has yet to be made out in many families, but it may be

1. Pinnate
   - (a) unifoliate
   - (b) acropetal and webbed
   - (c) basipetal and webbed
2. Palmate
   - (a) unifoliate
   - (b) webbed
3. Phyllodic from any of the other forms

The acropetal pinnate leaf, however, has a uniform construction. It bears evidence of having been derived from a branching system of unlimited growth, and modern genera show how it has been transformed through limitation of growth, webbing, and basipetal construction into the simple form.

To suppose that the simple leaf, as found today, should have evolved the apical growth and acropetal ramification in one plane of the pinnate leaf, and have carried this on to a high degree of ramification, and have done so not merely in many distinct families, but in many tribes and distinct genera within a family, without any deviation, is to maintain a belief in orthogenesis to the extent of special creation. If such orthogenesis be supposed to have pursued the course of the pinnate venation of the simple leaf, then why has the simple leaf this venation? That is the crux. However modified the leaf may be, the pinnate venation is, so far as anyone has yet discovered, always the result of acropetal growth. I can find no answer other than that the pinnate venation is the remains of the primitive acropetal construction of the leaf.

It is this point which prevents me from accepting Troll's interpretation. He proposes that the acropetal pinnate leaf has been derived from the basipetal through prolongation of apical growth. For him, the Angiosperm leaf is primarily an organ of limited growth, which has discovered on land apical growth. That is remarkable and contrary to botanical understanding of plant-form (Church, 1920). Moreover, on land, the basipetal leaf, with growing point so well protected, as in thistles and grasses and geophilous bulbs, all of which are the growth-forms of culminating families, is a perfection that would be folly on the part of orthogenesis to disturb. Troll's examples are taken mostly from the temperate floras of shrubby and herbaceous plants. In trees, such as *Gleditschia* and *Sorbus*, he finds acropetal growth, as occurs so abundantly in woody tropical families. His argument gives to *Anthyllis* (Papilionaceae), which is a temperate herb, a primitive leaf-form, and to the tropical mimosoid and caesalpinioid trees an advanced leaf-form, which is like pointing to *Myosurus* as the progenitor of the flowering plant. In several families, such as the Rosaceae, Umbelliferae and Compositae, Troll has shown that the leaf may be amphipetal (divergent), having a short period of apical growth and acropetal pinnation followed by a shorter or longer period of basipetal growth and pinnation. He interprets these leaves as on the upgrade to the acropetal. *A priori*, the series could be read in the opposite direction, and I consider that this is the only tenable inter-

pretation when one realizes that even the basipetally developed pinnae still develop acropetally themselves and have the pinnate venation. Where the leaf and pinna are truly basipetal, they have phyllodic venation, as in *Plantago* and monocotyledons. In other words, the very important detail of venation shows that the basipetally pinnate leaf is fundamentally acropetal, but that it has a basipetal development of pinnae imposed on it, as can be discovered in the amphipetal leaves of many families. The peltate leaf is, therefore, the ultimate term of reduction which fulfils webbing on a basipetally arranged, but acropetally developed, primordium. The amphipetal leaves are such a clue, discovered among living organisms, as shows the limitations of palaeobotany.

The Monocotyledonous Leaf

It is generally held that the pinnately divided palm-leaf has been derived from the simple liliaceous form of leaf. I do not find that this view is supported by botanical argument. Palms, themselves, point to the opposite conclusion. The pinnately divided palm-leaf is a basipetal structure, separating the leaflets by intercalary extension of the rachis. The palmately divided palm-leaf has no such extension, or comparatively little, for many palmate leaves have a short rachis supporting the lamina. The palmate form is, therefore, the telescoped, to be derived from the pinnate, as in dicotyledons. Now, the pinnate form may be webbed and undivided, or it may be once or twice pinnate: its leaflets may have one or several folds, and they may end in simple tapering points or be dilated into the fish-tail form: the leaflets may also have undulations along the sides. All these details need developmental explanation before the palm-leaf can be understood. I began such work in Singapore, but was unable to complete it. The following remarks, taken in conjunction with the recent contribution by Eames (1953), may stimulate others to investigate these tropical plants. An abundance of material is required, and axe, saw, scalpel, dissecting needle and microtome will successively be called into play.

The pinnae of the palm-leaf develop as folds in the lamina-tissue between the margin of the lamina and the rachis. The splitting of the folds into the leaflets is defined by anatomical structure, which leads to easy rupture on expansion, and there may also be chemical dissolution of the tissue. The pinnae are splaid apart and set to their characteristic angles by the swelling of the pulvinus at the base of the fold, where it joins the rachis. They are then spaced by the elongation of the rachis, which is, of course, basipetal. The mechanism is elaborate. In some palms, as *Pinanga* and *Areca*, the splitting develops at every third, fourth, fifth or seventh fold, though each has a pulvinus at the base, and thus their leaflets have as many longitudinal ribs or ridges. In a few palms there is no splitting, and the open lamina is continuous except at the edge where it has broken away from the marginal strip. Such entire, but pinnately constructed, leaves suggest the upgrade form intermediate between the entire, but plicate, monocotyledonous leaf, as seen in *Veratrum*, and the pinnately divided palm-leaf. In fact, they occur with no such primitive indication. Simple-leafed palms occur in such specialized genera as *Pinanga*, *Iguanura*, *Chamaedorea*, *Geonoma*, *Salacca*, *Teysmannia*, and *Verschaffeltia*, as well as in palmate-leafed genera as *Licuala*. In the first four genera, and in *Licuala*, which abound in species and which represent the herbaceous kind of palm, there are transitions from the slender, simple-leafed forms to the more robust with highly divided fronds. Are we to suppose that these genera, which are by no means closely related, show among their living species the evolution of the palm-leaf, which had already occurred by the Cretaceous? It is as absurd as to suppose that *Plantago* shows the evolution of the dicotyledonous pinnate leaf. These small palms, when judged by other palms, are seen to be the diminutive, herbaceous undergrowth forms of their genera, in which simplified inflorescence, flower, fruit and leaf conform with the slender stem. The undivided leaf-form is, in fact, the juvenile leaf-form which the palm-herb retains in conformity with its physiological precocity, just as

most herbs resemble precociously matured sapling stages of the more robust forms of their genera. *Salacca* is a good example (Furtado, 1949).

This explanation of the simple leaf in palms applies, also, to the Cyclanthaceae, which are often regarded as something upgrade and intermediate between Liliaceae and palms. Rather are they another example of the palm-herb, though of proto-palmaceous origin. The two families, Cyclanthaceae and Palmae, show the ancient nature of the pinnately folded monocotyledonous leaf, and the derivative nature of the unsplit leaf (see Eichler, 1885).

Caryota is the one genus with doubly pinnate leaves. The primary folds of the primordial leaf become themselves folded, so that the lamina is laid down in these secondary folds, while the primary become the branches of the rachis. A pulvinus occurs at the base of each primary and secondary fold. But the secondary folds have an irregular junction with the marginal strip and, on breaking away, form the fish-tail ends of the leaflets. This character occurs in many palms of the *Caryota*-alliance and also in the distinct group of lepidocaryoid palms. It seems always to be connected with the secondary folding of the primary folds and is, therefore, a relic of the double pinnation of palms. It is extremely unlikely that all these fish-tailed genera are now independently and orthogenetically evolving the doubly pinnate leaf by the same means. Such argument would imply that the doubly pinnate leaf were the most highly evolved and that in the next millennium there might be more. The successful genera, measured in numbers of species, are those with the sub-herbaceous forms, that I have mentioned, and such as *Calamus*, which is the " Rubus " of palms. It is far more likely from the palm point of view that *Caryota*, with its few species, persists as a relic of doubly, and even trebly, pinnate palm-ancestors. A critical genus in which to study the derivation of the simply pinnate condition would be *Arenga* with its undulate and toothed leaflets. A detail, too, worth noting, as others have observed, is that the first fold to develop in the primordial palm-leaf is followed by other folds developed *acropetally* as well as basipetally, though the basipetal are far more numerous. Nevertheless, there is clear indication that the palm-leaf is to some extent amphipetal, retaining a small and vestigial amount of acropetal growth.

Further simplification of the palm-leaf would lead to the plicate basipetal leaf and then to the flat phyllode, as an even more juvenile form without folding. The plicate leaf seems to me to be as interesting a feature as the winged seed.

Evidence that the simple monocotyledonous leaf is not at all simple, but has a long ancestry of progressive simplification, comes also from the Araceae, which Troll has investigated, and from the Taccaceae. In these families the pinnate leaf develops its lobes extra-marginally, as in dicotyledons, so that there is no marginal strip to be shed on expansion, and it reduces within the limits of single genera to the simple, undivided state with parallel venation, e.g. *Philodendron*, *Anthurium*, *Scindapsus* and *Tacca*. Whether any aroid leaf is entirely acropetal, I do not know, but it is a matter worth investigating. Many, however, as Troll has shown, are amphipetal with a considerable amount of acropetal growth, while others are mainly or entirely basipetal, whether pinnate, palmate or simple. And these simple leaves are not plicate, but convolute and lead to another kind of simple monocotyledonous leaf. I find it strange that this resemblance between *Tacca* and the aroids has received no attention. It singles them out from other monocotyledons.

The great size of the palm-leaf has caused its ommission from orthodox botany, and the discovery that the pinnae have an intra-marginal origin has encouraged the assumption that the leaf is a curiosity of no consequence to general botany. When viewed from the tropics, however, in company with pinnate aroids and plicate-leafed orchids and grasses, it becomes a central problem in monocotyledonous science. Its success depends to no small extent on the pulvini. Now the pulvinus is widespread in monocotyledons. It not only opens the palm-leaf, but it spreads the spines and displays the

branches of the inflorescence, as it does in Liliaceae, Gramineae, Cyperaceae, and Bromeliaceae, for instance: it opens the grass-floret and in a basipetally extended tissue it unrolls the rolling grass-leaf. I wonder how much the basipetal success of the monocotyledon has depended on the pulvinar mechanism, which is mainly lacking in dicotyledons. Its importance has been realized by van der Pijl (1952): it recalls to me the parenchymatous mechanism of the arillate follicle.

The Gymnospermous Leaf

Much of the durian theory applies also to gymnosperms, the theory of which suffers from rather too academic an outlook. The basipetal tendency, consummated in the angiosperms as the leaf that does not expose the growing point, has developed widely in gymnosperms. *Welwitschia* is the obvious and academic instance, but it is as blatant in the sharp-tipped coniferous needle, as it is in the species of *Araucaria*, *Agathis* and *Podocarpus* with parallel veins (Laubenfels, 1953). By analogy, the cordaitalean leaf was phyllodic. The coniferous leaf, with two vascular bundles, seems to have been derived from a bifurcate leaf, which indicates some acropetal development (Florin, 1951). This, again, seems to have come from a repeatedly dichotomous, yet more acropetal leaf, of the *Baiera*-type. This dichotomous leaf can be webbed, as in *Ginkgo*, and it would seem that the cordaitalean leaf is a phyllode of the webbed *Ginkgo*-type, which can be seen in miniature in the bud-scales of *Ginkgo*. The cone-scales, too, are basipetal, with vestigial acropetal tips, as on the palm-leaf: it is this basipetal growth which causes the rather academically described adnation. Possibly the leaflets of cycads with parallel veins are also basipetal, as their cone-scales certainly are. Basipetal growth, doing away with apical and exposed growing points, has even greater survival value on land than in the sea. It has led ultimately to very simple and spuriously primitive appendages, which only comparative ontogeny can elucidate. In less derivative states, limitation of apical growth of the acropetal leaf has led to all manner of transference of growth substances so that a multitude of parallel leaf-forms have been evolved in different families and even in different genera of the same family.

Arillate Families . . . An Omission

Gonystylidaceae: *Gonystylus* (see Airy Shaw, 1950).

Summary

Further evidence is brought forward to prove that the primitive arillate follicle must have been large, many-seeded, parenchymatous and fleshy, and spiny. The winged seed is regarded as an immediate derivative of the exarillate. Winged seeds and spiny fruits are guides to phylogenetic research.

Comparative morphology indicates that the highly pinnate, acropetal leaf must have been precursor of both dicotyledonous and monocotyledonous leaves. Simple leaves are the most varied in morphological aspect. The basipetal scale or needle is the most derived form in angiosperm and gymnosperm. The palm-leaf is examined: it is concluded that the plicate leaf leads to the flat phyllode.

Gonystylus was omitted from the previous list of arillate families.

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THE GAMETOPHYTE OF FIVE SPECIES OF *PLATYCERIUM*

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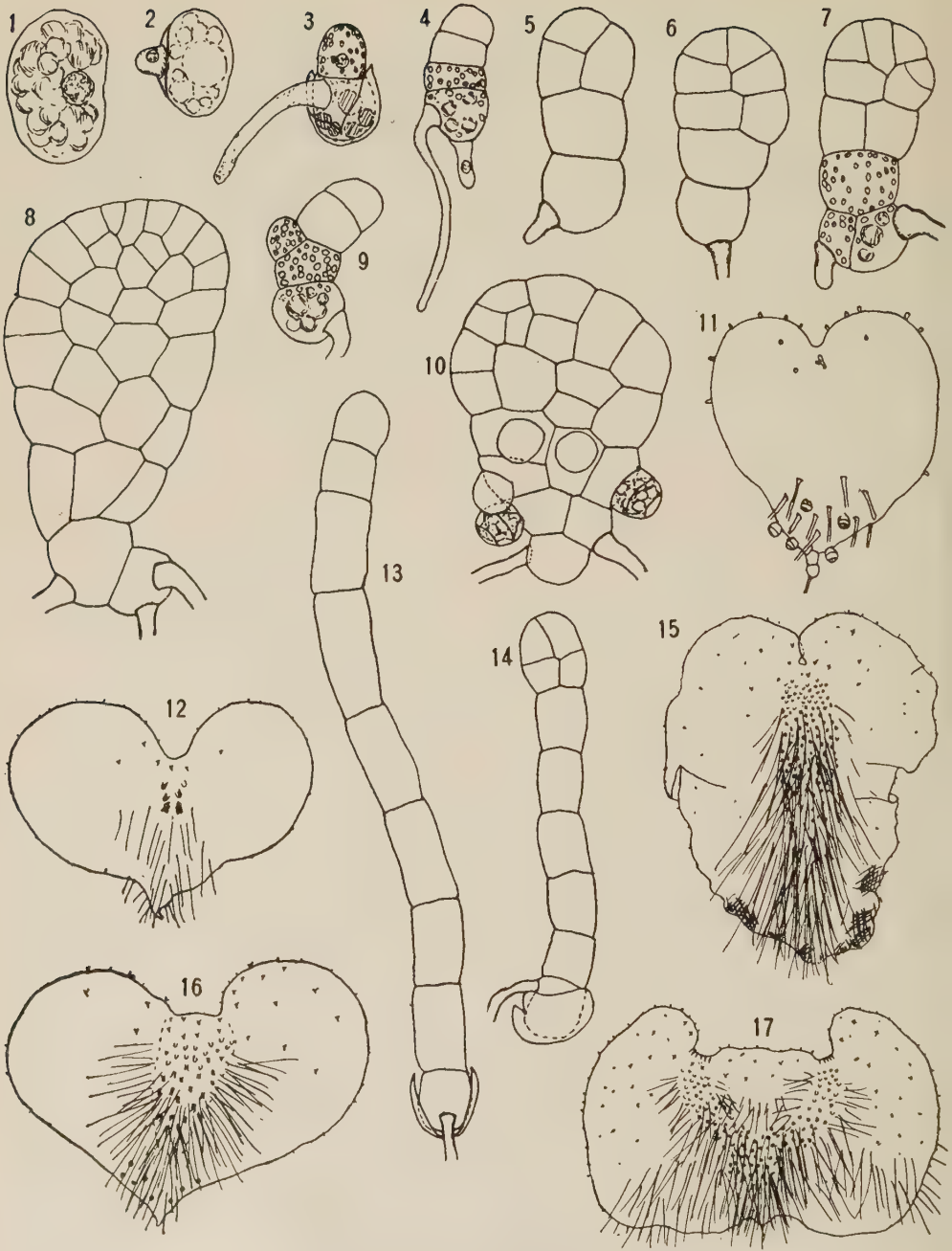
Most references to the gametophyte of *Platycerium* are based on the work on *P. grande* by Bauke (1878, 1880) left unfinished at his death, and that of von Straszewski (1915) which deals primarily with the sporophyte of the same five species treated in this present paper. Schmelzeisen (1933) included *P. hillii* and *P. willinkii* in his series of developmental studies of fern gametophytes, and Orth (1936) studied germination of the spore of *P. alcicorne*.

In our work we have investigated the following species: *P. alcicorne* (Willd.) Desv. [*P. stemaria* (Beauv) Desv.] from Mount Holyoke College and from the Brussels Botanic Garden; *P. grande* from Smith College, Northampton, Mass.; and three species for which we are indebted to the Brussels Botanic Garden — *P. bifurcatum* (Cav.) C. Chr., *P. hillii* Moore, and *P. willinkii* Moore.

The spores of *Platycerium* are bilateral with a short inconspicuous ridge, yellow from many oil globules, with a smooth transparent coat faintly brownish in colour. The spores of *P. alcicorne* average in size 58 × 35 μ (Fig. 1), and those of the other species are of approximately the same size. Germination occurred in five to seven days on distilled water with a lengthwise split of the spore coat through which protruded a rhizoid brownish in colour from its very first appearance (Fig. 2); this colour of the first rhizoid is unusual in ferns and was noted as such by both Bauke and von Straszewski. As germination proceeds the small oil drops coalesce into larger drops; chloroplasts may be seen in the first prothallial cell but often do not appear until the formation of the second prothallial cell (Fig. 3) while the basal cell is still yellow with oil globules. By the time there are two to four green cells in the filament the basal cell may bear two conspicuously brown rhizoids (Fig. 4). Oil globules may be seen in the basal cell



FIGS. 1-17 — Spore and stages in development of gametophyte. Fig. 1. Spore. × 350. Fig. 2. Germination, 5 days. Fig. 3. 8 days. Fig. 4. 10 days. Figs. 5-7. 16 days. × 225. Fig. 8. 15 days. Fig. 10. 33 days. Fig. 11. Thallus with antheridia and one branched hair, 42 days. × 37. Fig. 12. Thallus with archegonia and branched hairs, indicated by checks, 2 months. × 21. Figs. 13, 14. Filaments germinated in sporangium, 33 days. × 190. Fig. 15. 9 months. × 8. Fig. 16. 3½ months. × 13. Figs. 17. 5 months. × 8. Figs. 1-9, 12-15. *P. alcicorne*. Figs. 10, 11, 16, 17. *P. grande*.

even when the plate consists of 10-15 cells (Fig. 7).

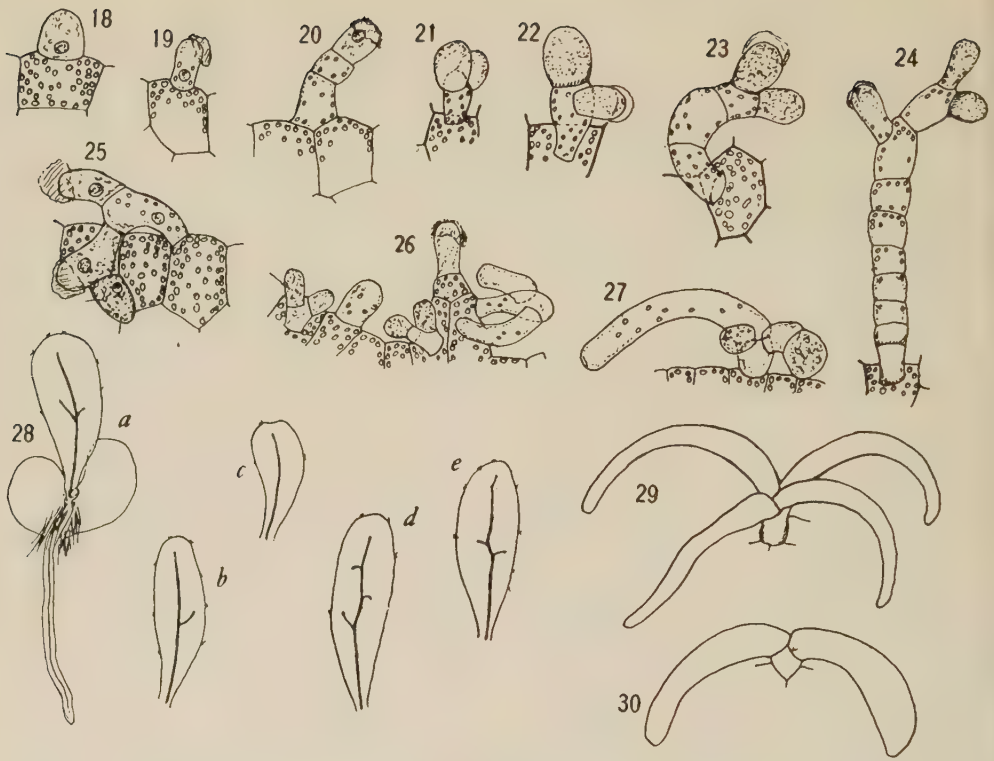
A filament is formed on germination, the length of which depends on the amount of light and space. Both Bauke and von Straszewski mentioned a tendency which they found in the *Platycerium* prothallus to branch in the filamentous stage in the second or third, or even the first cell. In our cultures this type of thallus has been associated with unfavourable conditions. Gametophytes with adequate light rarely showed branching of the filament or lobing of the plate, but developed symmetrically in both early and late stages. In cultures 15-16 days old, when the prothalli were mostly in the stage of those in Figs. 5-7, there would be only one or two irregular thalli (Fig. 9) in a collection of 40-50 mounted in water on a slide.

If conditions are favourable about two weeks after the spores are planted, the filament while still short begins to broaden by longitudinal and oblique divisions, but if the filaments are crowded, they may elongate considerably (Figs. 13, 14). In our material the growth of the filament was not stopped by a terminal papillate hair as described by Bauke for *P. grande*. Von Straszewski found many variations in his material with branched filaments but no uniformity in regard to terminal hairs. Orth found filaments with terminal papillate hairs and also those in which the terminal cell formed an apical cell. Schmelzeisen did not find terminal papillate hairs in *P. hillii* and *P. willinkii*. The gametophyte of *Platycerium* develops hairs sooner or later, and it is to be expected that under some conditions — and perhaps in some strains — the terminal cell of the filament will end in a papillate hair, but this habit is apparently the exception.

An apical cell appears as the thallus continues to broaden by longitudinal and oblique divisions (Fig. 8). A longitudinal or oblique division in the basal cell is not uncommon; in such cases the cell with the primary rhizoid retains reserve oil but the other usually has none. The apical cell undergoes a considerable number of divisions and the thallus assumes a cordate form before the apical cell is replaced by a marginal meristem. In all our species the thallus became broadly cordate or reniform at an early age (Figs. 12, 16). It usually remained flat for some months, but if the culture became crowded as the thalli developed, the wings showed a tendency to become lifted. The midrib never became very heavy and on old gametophytes at five or six months it was not more than five cells thick (Fig. 64). In gametophytes in culture for 6-12 months, the posterior region of the thallus turns brown and dies even though the anterior region remains green and bears functioning archegonia (Fig. 15); this habit is common in gametophytes of higher ferns which mature early. Occasionally we found a forking of the midrib caused by the development of a region of permanent tissue in the middle of the apical meristem; this region may elongate into a lobe or form a broad region of permanent tissue one cell thick between two cushions (Fig. 17). No real branching of the thallus was seen.

The rhizoids of young gametophytes of *Platycerium* are conspicuous for their colour, but those of old thalli not only for their colour but also for length, stoutness and great abundance. They develop not only on the midrib but on the wings (Figs. 12, 17); there may also be marginal rhizoids in considerable numbers on the posterior parts of the wings.

The gametophyte of *Platycerium* is notable for the abundance of hairs, not only short blunt unicellular hairs on the margin and surfaces (Figs. 18, 19) and the less common simple multicellular hairs (Fig. 20), but chiefly for the multicellular branched hairs (Figs. 21-27). In our cultures the first to appear were simple marginal hairs when the cultures were four or five weeks old. Branched hairs were found in cultures six weeks old (Fig. 11) before the thallus had developed a midrib; this was the case in *P. alcicorne* and *P. grande*, but we did not follow so close a series in the other three species. The branched hairs increase in number after the formation of the midrib and are most numerous on or near the midrib and on the wings near the notch (Figs. 15-17). They are found

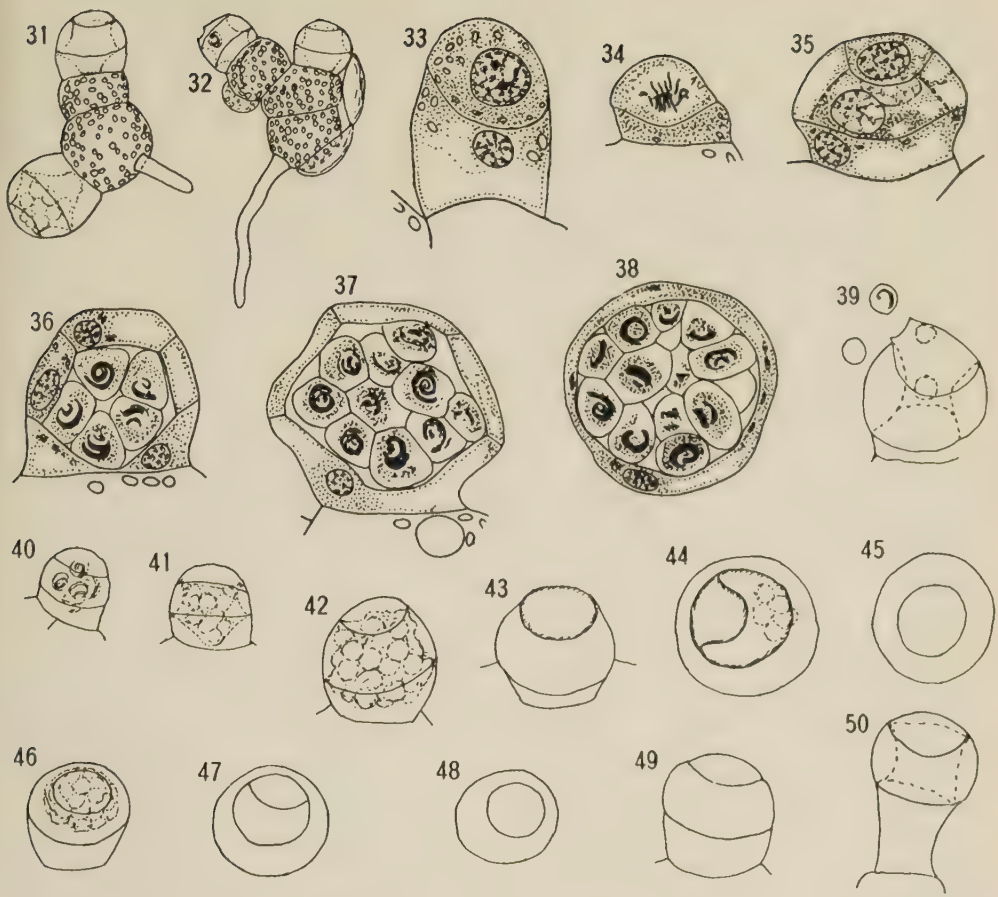


FIGS. 18-30 — Figs. 18-20. Simple hairs on margin of thallus. × 225. Figs. 21-24. Branched hairs from ventral surface. × 225. Figs. 25-27. Branched hairs from margin. Fig. 28, *a*. Gametophyte with young sporophyte; *b-e*, primary leaves from four other sporophytes. × 4. Figs. 29, 30. Branched hairs from primary leaf. Figs. 18, 21, 22, 28-30. *P. alcicorne*. Figs. 19, 20, 25, 26. *P. grande*. Fig. 24. *P. hillii*. Figs. 23, 27. *P. bifurcatum*.

on both dorsal and ventral surfaces, and on older thalli near the margin. In our cultures the branched hairs were most abundant on *P. grande* and *P. hillii*, but the same range in form and size was found on all species. The branched hair may consist of only three or four cells, but on old thalli it may have 10-12 or even more. The terminal cell of each branch usually becomes glandular and eventually turns brown; the stalk usually retains some chlorophyll and the walls remain colourless or the base may become slightly brownish. Marginal branched hairs were found on old gametophytes; occasionally a marginal hair is found in which one branch develops as a long curved cell which is not glandular (Figs. 26, 27). These are similar to the hairs on young leaves (Figs. 28-30), as noted by von Straszewski, but those on the gametophyte unlike the more or less stellate hairs on young leaves are usually of a mixed type with one or more short glandular branches as well as the longer curved colourless branches.

Antheridium

Under certain conditions antheridia may appear very early on small male gametophytes consisting of 2 to 4 green cells, in cultures 20-24 days old (Figs. 31, 32). When gametophytes developed as symmetrical plates we found antheridia near the base on those four weeks old usually on marginal cells (Figs. 10, 11); later they were more numerous on the ventral surface. They may develop also on more or less



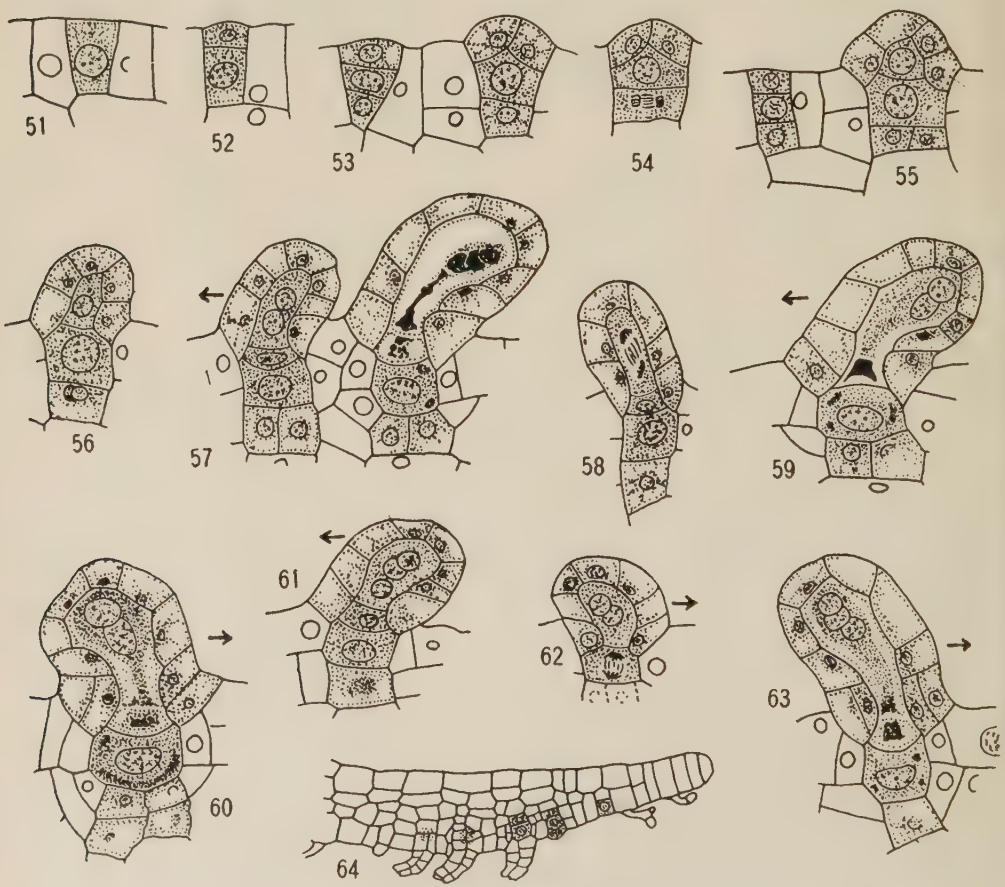
FIGS. 31-50 — Antheridium. Figs. 31, 32. Prothalli with antheridia, 24 days. × 225. Figs. 33-35. L.S. of stages in development. × 700. Figs. 36, 37. L.S. matu re antheridia. × 700. Fig. 38. T.S. mature antheridium. ×700. Fig. 39. Dehiscence. Figs. 40-43, 46,49, 50. Surface views from side. × 300. Figs. 44, 47. Top view, two cap cells. × 300. Figs. 45, 48. Top view, one cap cell. × 300. Figs. 31, 33-36, 40-46. *P. alcicorne*. Figs. 32, 37, 38, 47, 48. *P. grande*. Fig. 46. *P. bifurcatum*. Fig. 49. *P. willinkii*. Figs. 39, 50. *P. hillii*.

irregular thalli one cell thick or on regenerated branches. Antheridia were produced in *Platycerium* rather less abundantly than in most of the higher ferns which we have in culture. Many young gametophytes, which were just beginning to bear archegonia, had a few small antheridia close to the base; several of these appeared to contain not more than 8 sperms (Figs. 36, 40), but others had probably 16. If the thallus continues to develop antheridia for a considerable period it may bear 20-40 before archegonium production begins, and the later antheridia are larger in size (Figs. 37, 38). We found the antheridium to be of the type characteristic of the higher ferns, as indicated by the developmental series (Figs. 33-37), with a wall consisting of a basal or funnel cell, a ring cell and a cap cell (Figs. 31, 32, 40-43, 46, 49, 50). Bauke, who found antheridia on small male prothalli only, thought that *Platycerium* might possibly be dioecious; he described some antheridia as one-storied (einstöckig). Von Straszewski described the antheridium as having a divided cap cell and gave a figure of it in *P. grande*. Schmelzeisen ascribed a divided cap cell to *P. willinkii* and *P. hillii* also, but he

went into no detail concerning frequency of occurrence and gave no figures; he considered it an evidence of connection of *Platycerium* with the Cyatheaceae. Our investigations do not support the sweeping statements of von Straszewski and Schmelzeisen. We found that the great majority of antheridia seen in the five species had undivided cap cells (Figs. 35-37, 40-43, 45, 46, 48-50), and that the divided cap cell was of exceptional occurrence and was found only on large antheridia (Figs. 44, 47). The antheridium is in general globular (Fig. 42), or slightly elongated (Figs. 43, 46, 49). One example of an antheridium with an elongated base was seen on an old thallus (Fig. 50). In none of the material under observation was a dislodged cap cell seen; on dehiscence sperms were seen discharged through a pore at the side of the cap (Figs. 32, 39).

Archegonium

The archegonia began to appear when the gametophytes were a little less than two months old. They are not found as close to the apex as is usual in ferns; in the region between the notch and the



FIGS. 51-64 — Archegonium. Figs. 51-63. Stages in development. Fig. 57. Adjacent archegonia; left, axial row complete with two neck canal nuclei; right, mature with three neck canal nuclei. The arrow points towards the notch. Fig. 58. Division of neck canal nucleus. Figs. 59, 60. Mature archegonia. Fig. 61. Axial row complete with three neck canal nuclei. Fig. 62. Division of central cell. Fig. 63. Mature archegonium. Figs. 51-63. × 320. Fig. 64. L.S. thallus, archegonia and branched hairs, 5 months. × 75. Figs. 51-53, 55-57, 59, 61, 63, 64. *P. grande*. Figs. 54, 58, 60, 62. *P. alcicorne*.

archegonia there are simple and branched hairs (Figs. 15, 16, 64). The archegonia appeared in some cases on thalli which had borne antheridia (Fig. 16), but often on those which had not (Fig. 12). The production of antheridia ceases when archegonium production begins, and no young antheridia were found on archegoniate prothalli. In the case of old thalli the heavy brown rhizoids make such a dense mat that it is not possible to tell whether or not there have been antheridia at the base.

The development of the archegonium takes place in the usual manner (Figs. 51-60). The ventral canal cell is formed sometimes before and sometimes after the division of the neck canal nucleus (Figs. 58, 62). The basal cell divides early (Figs. 54-56) and its products are easily identifiable during the entire development of the archegonium. In *P. alcicorne*, *P. grande* and *P. hillii* three nuclei were found as commonly as two in the neck canal (Figs. 61, 63); only binucleate neck canal cells were observed in *P. willinkii*. Archegonia of both types appear functional. The neck of the mature archegonium has usually five and four cells on the long and short sides of the neck respectively (Figs. 57, 60) and bends somewhat strongly away from the notch (Figs. 57, 59, 61, 63, 64). It is somewhat bulbous at the tip (Figs. 57, 59, 60); the lowest cells — those near the venter — divide at maturity (Figs. 60, 63). The usual ventral jacket is formed when the axial row of the archegonium is complete. The cytoplasm of mature eggs contains localized deeply staining material which seems to be of vacuolar nature.

Fertilization occurred occasionally in cultures of *P. alcicorne* and P. *grande* resulting in the development of sporophytes (Fig. 28, *a*). The primary leaf showed considerable variation in venation, but there was always a well-defined midrib (Fig. 28, *a-e*).

Discussion

This investigation has brought out no evidence from the gametophyte to justify the family Platyceriaceae (Ching, 1940), and no reason to question the place of the genus *Platycerium* among the more primitive members of the Polypodiaceae as defined by Copeland (1947). The gametophyte is of the type well known in the higher ferns, agreeing in spore germination, early development and mature form of the gametophyte, and in type of sex organs. There are, however, certain special features in the gametophyte: the brown colour of the first rhizoid from its earliest appearance, the production of branched multicellular glandular hairs, and the occasional division of the cap cell of the antheridium.

The character of the rhizoid of *Platycerium* has not been noted in any other fern, but the developmental history of relatively few ferns is known. Multicellular hairs have been described for other genera of Copeland's Polypodiaceae: *Drynariopsis* (Klein, 1881), *Dendroglossa* (Schumann, 1915), *Belvisia*, *Phlebodium*, *Pyrrosia* and *Pessopteris* (Stokey, 1951). Two genera with multicellular hairs have been reported for the Aspidiaceae: *Stenosemia aurita* (Sw.) Presl. by Schumann and *Aspidium moorei* (Hk.) Diels [*Cionidium moorei* (Hk.) Moore] by Köhler (1920). We have found a few cases in *Cyclopeltis crenata* Fée but of rather a different type from those in *Platycerium*. The divided cap cell of the antheridium has been reported by Schmelzeisen, Schlumberger (1911), and others as of occasional occurrence in *Woodsia*, *Diacalpe*, *Adiantum*, *Cryptogramme*, *Drynariopsis* and *Blechnum*. These examples are so scattered that it is difficult to know how to evaluate their significance. Von Straszewski and Schmelzeisen both suggested that the multicellular hairs of *Platycerium* indicate a connection with the Cyatheaceae. This does not seem probable in view of the difference in their origin and type; the short multicellular glandular hairs of the Gleicheniaceae and the larger, more abundant, bristle-like hairs of *Cyathea* arise from special initials, each cut as a wedge-shaped cell from the anterior face of cell on or near the midrib and not far from the apex (Stokey, 1930, 1950), while the branched multicellular hairs on *Platycerium* and the other genera mentioned

above do not arise from special initials but from near the centre of any cell of the midrib, wing or margin.

The question of the relationship of *Platycerium* to *Cheiropleuria* is discussed in another paper (Stokey & Atkinson, 1954). It has been generally associated with *Cheiropleuria* although the connection is not considered to be close (Christensen, 1938; Holttum, 1949; Copeland, 1947). The great difference between their gametophytes indicates that the connection is very remote indeed.

Summary

In an investigation of the gametophyte of five species of *Platycerium* they are found to be of the type common in the higher ferns, broadly cordate with a midrib not more than five cells thick. They bear archegonia when less than two months old on thalli which may or may not have borne antheridia at the base. Antheridia may develop on young prothalli which consist of only two or three cells. The special features are: the brown colour of the first rhizoid on emerging from the spore coat, multicellular branched glandular hairs on midrib, wings and margin, and an occasional antheridium with a divided cap cell.

Part of the work of the senior author was carried on at the Marine Biological Laboratory, Woods Hole, Mass.

The junior author wishes to thank the Biology Department of Amherst College for many courtesies and certain technical assistance.

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CONTRIBUTION TO THE EMBRYOLOGY OF *DENDROPHTHOE* MART.

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The parasitic Loranthaceae are very peculiar in their mode of life, floral structure and reproduction. Although considerable work has been done, the morphology and embryology of many forms is still very inadequately known. Several genera are being studied in this laboratory: *Amyema, Arceuthobium, Barathranthus, Helicanthes, Helixanthera, Korthalsella, Lepeostegeres, Lysiana, Nuytsia, Phoradendron, Scurrula, Tapinanthus, Tapinostemma, Taxillus* and *Tolypanthus.*

The literature on the sub-family Loranthoideae has been reviewed by Singh (1952), and Maheshwari and Singh (1952) in their papers on *Dendrophthoe falcata* and *Macrosolen cochinchinensis* respectively. Other recent publications are by Maheshwari and Johri (1950) on *Helixanthera ligustrina*, Pienaar (1952) on *Loranthus rubromarginatus* (=*Tapinanthus rubromarginatus*), Smart (1952) on *Tupeia antarctica*, Narayana (1954) on *Lysiana exocarpi*, Johri and Agrawal (1954) on *Helicanthes elastica*, and Dixit (1954) on *Amyema congener, A. pendula, A. preissii* and *A. miquelii.*

This paper deals with some points on the embryology of *Dendrophthoe neelgherrensis* (W. & A.) Van Tieghem (=*Loranthus neelgherrensis* W. &. A., *L. pyranthus* Wight., *L. nilgherrensis* Benth. & Hook. and *Dendrophthoe nilgherrensis* Van Tieghem) and the endosperm and embryo of *D. falcata* (L.f.) Ettingsh. Among previous studies the most important are by Singh (1952) on *D. falcata* and Rauch (1936) on *D. pentandra.* So far there has been no work on *D. neelgherrensis.*

Material and Methods

Preserved material of *D. neelgherrensis*, collected by Dr. B. M. Johri from Kodaikanal (Madras State) in January, 1951, was kindly passed on to me for investigation. Further collections were made by me in January 1953, from the Peradeniya and Hakgala Botanical Gardens (Ceylon). Material of *D. falcata* was obtained from several places: Ernakulam, Bangalore, Kolar, Hyderabad-Deccan, and Delhi in India; and Peradeniya and Hakgala in Ceylon. That from Ernakulam and Hyderabad was very kindly provided by Prof. T. S. Sadasivan (Madras) and Dr. Vaheeduddin (Hyderabad).

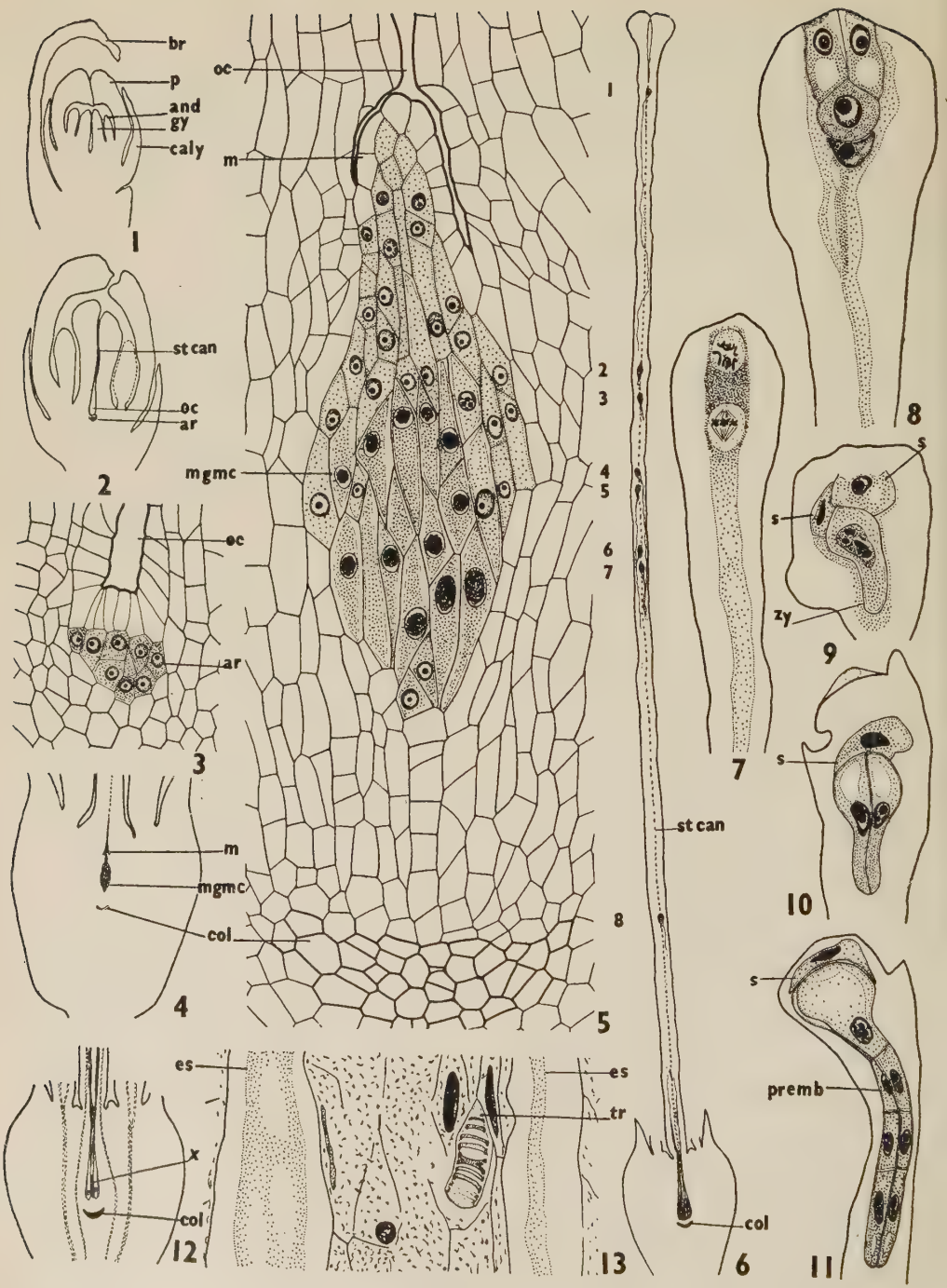
Formalin-acetic-alcohol was used for fixation in all cases. The fruits were softened in 5 per cent hydrofluoric acid (diluted in 70 per cent ethyl alcohol) for 10 days and run up through the tertiary-butyl alcohol series. Sections were cut 5-15 μ thick and stained in iron-haematoxylin and in safranin-fast green. The latter combination gave better results. Dissections of embryo sac, embryo and endosperm in lactophenol proved very helpful.

Observations

Dendrophthoe neelgherrensis

MEGASPOROGENESIS — Fig. 1 shows a longitudinal section of a young bud. The calyculus, perianth and androecium have already been differentiated. The stylar canal is continuous with the ovarian cavity. In older buds (Fig. 2) the stylar canal becomes narrow while the base of the ovarian cavity broadens. There is no placenta nor any ovules in the usual sense.

From the floor of the ovarian cavity differentiates a multicelled sub-epidermal archesporium (Fig. 3). The epidermal cells too stain quite densely and somewhat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FIGS. 1-13.

simulate the archesporial cells lying below them which soon divide to give rise to a massive sporogenous tissue. Since no parietal cells are cut off, the sporogenous cells function directly as megaspore mother cells. As they elongate, the base of the ovarian cavity is pushed up in the form of a conical projection or " mamelon " (Figs. 4, 5). This is very similar to the structure which Dixit (1954) has observed in *Amyema*.

Slightly below the elongating megaspore mother cells, 3-5 layers of parenchymatous cells become thick-walled and differentiate into a cup-shaped collenchymatous pad. In later stages the cells become thick-walled and pitted (Fig. 15) and are closely pressed against each other. Several of them become arrested in their growth and degenerate. As a rule the basally situated mother cells are healthier than the upper ones. They undergo the usual meiotic divisions and give rise to linear tetrads of megaspores. Usually only the chalazal[1] megaspore of each tetrad enlarges further. By this time the conical projection or " mamelon " has degenerated, but its remains can be recognized for a long time.

FEMALE GAMETOPHYTE — Several embryo sacs develop simultaneously and protrude upwards into the style up to different heights (Fig. 6). Many embryo sacs were seen with two nuclei[2] at the upper end (Fig. 7) which later divide to form the quartet responsible for organizing a normal egg apparatus and the upper polar nucleus (Fig. 8). The lower polar nucleus and two antipodals (the upper of these is binucleate) organize in the basal part of the embryo sac. In later stages the lower end of the embryo sac forms a caecum which extends downwards as far as the collenchymatous pad, while the antipodals are left behind *in situ*. The formation of a caecum has not been reported previously in the genus *Dendrophthoe*.

In *D. falcata*, according to Singh (1952), the egg apparatus is organized at a time when embryo sacs are still in the lower part of the style and that further elongation takes place only after the organization of the egg apparatus. In *D. neelgherrensis* I observed many embryo sacs which had already reached more than two-thirds of the length of the style, but their tips showed only two nuclei. This clearly shows that the last nuclear division in the maturation of the embryo sac and the differentiation of the egg apparatus may take place at almost any level in the style. The embryo sacs (usually 7-10 in number) follow a tortuous course and are often twisted around one another. At first they grow in the stylar tissue but eventually their tips come to lie in the stylar canal.

A feature of special interest is the occurrence of isolated tracheids inter-

1. Since there is nothing which can properly be called micropyle or chalaza, the term chalazal is used here in the same sense as lower or basal.

2. Since the embryo sacs are extremely long and twisted, it is almost impossible to trace any of them from top to bottom in sections. However, the lower ends of such embryo sacs already showed the antipodals. It is, therefore, likely that, as in *Macrosolen cochinchinensis* (Maheshwari & Singh, 1952), after the 4-nucleate stage, the two chalazal nuclei divide first and then the micropylar so that a 6-nucleate stage intervenes between the 4- and 8-nucleate stages.

←

FIGS. 1-13 — *Dendrophthoe neelgherrensis* (*and*, androecium; *ar*, archesporium; *br*, bract; *caly*, calyculus; *col*, collenchymatous pad; *es*, embryo sac; *gy*, gynoecium; *mgmc*, megaspore mother cells; *oc*, ovarian cavity; *p*, perianth; *m*, mamelon; *pr emb*, proembryo; *s*, synergid; *st can*, stylar canal; *tr*, tracheid; *zy*, zygote). Fig. 1. L.S. 1 mm. long bud. × 30. Fig. 2. L.S. bud at archesporium stage. × 30. Fig. 3. Part of same enlarged to show multicelled archesporium. × 298. Fig. 4. L.S. ovary at megaspore mother cells stage. × 20. Fig. 5. Part of same magnified to show megaspore mother cells and " mamelon ". × 298. Fig. 6. Diagram to show the height to which the embryo sacs grow in the style; 8 embryo sacs are present. × 7. Fig. 7. Upper part of an embryo sac with two nuclei in division. × 298. Fig. 8. Upper end of 8-nucleate embryo sac. × 298. Fig. 9. Elongating zygote. × 298. Fig. 10. First division of zygote. × 298. Fig. 11. Biseriate proembryo. × 298. Fig. 12. Ovary of open flower to show position of a tracheid at place marked X. × 12. Fig. 13. Region marked X in Fig. 12 enlarged to show the details. × 500.

FIGS. 14, 15 — *D. neelgherrensis* (*d*, degenerating tissue between the embryo sacs; *c end*, composite endosperm; *col*, collenchymatous pad; *p en*, primary endosperm nucleus; *pr emb*, proembryo). Fig. 14. L.S. central portion of ovary showing primary endosperm nucleus in the lower part of the embryo sac on the right side, the other embryo sac shows cellular endosperm. × 297. Fig. 15. Same, later stage showing two proembryos; composite endosperm has already formed in the basal part (reconstructed). × 297.

spersed in the degenerating mass of megaspore mother cells, tetrads and embryo sacs (Figs. 12, 13). Dixit (1954) has recently reported that in *Amyema miquelii* some of the megaspore mother cells become transformed into tracheids which persist up to the formation of the endosperm. In *Dendrophthora* (Viscoideae) York (1913) reports a pad of tracheids instead of the usual collenchymatous pad.

Endosperm — Triple fusion was not observed, but it is presumed that it occurs in the upper part of the embryo sac. The primary endosperm nucleus migrates to its lower end which is situated in the ovary (Figs. 14, 15). The earliest stage in my material showed a 4-celled condition. Cell formation continues upwards from the base and results in a 4-seriate endosperm tissue. During the further growth of the different embryo sacs in an ovary, the intervening tissue is crushed and digested, and ultimately all the endosperms fuse to form a single structure (Fig. 15). The base of the composite endosperm forms an annular ring around the collenchymatous pad. As in *D. falcata*, it grows down at a more rapid rate on one side and pushes the collenchymatous pad to a lateral position.

Embryo — The first division of the zygote is vertical (Figs. 9, 10). This is followed by transverse divisions resulting in a biseriate proembryo which differentiates into an upper suspensor part and a lower embryo proper (Fig. 11). Repeated transverse divisions and elongation of the suspensor cells push the proembryos to the base of the style and finally into the ovary where they make their way through the endosperms and grow down to the collenchymatous pad (Fig. 15). At this stage the terminal cells multiply rapidly to form a mass of densely staining cells. Though 2-3 club-shaped embryos develop in an ovary only one grows to maturity. There are two unequal cotyledons which enclose the plumule and become fused at the tip.

The suspensor becomes highly coiled and later stages suggest that the embryo is pulled up slightly so that it comes to lie more or less in the centre of the endosperm.

Fruit — As the ovary matures, the endosperm extends downwards as well as laterally, and consumes the parenchymatous cells around it until it comes in contact with the vascular tissue (Fig. 16). The conical base is solid but the upper part has a tubular cavity into which fits the embryo.

The mature embryo is 3 mm. long by 1 mm. broad. The radicular end projects outside the endosperm and shows many papillate processes (Fig. 17).

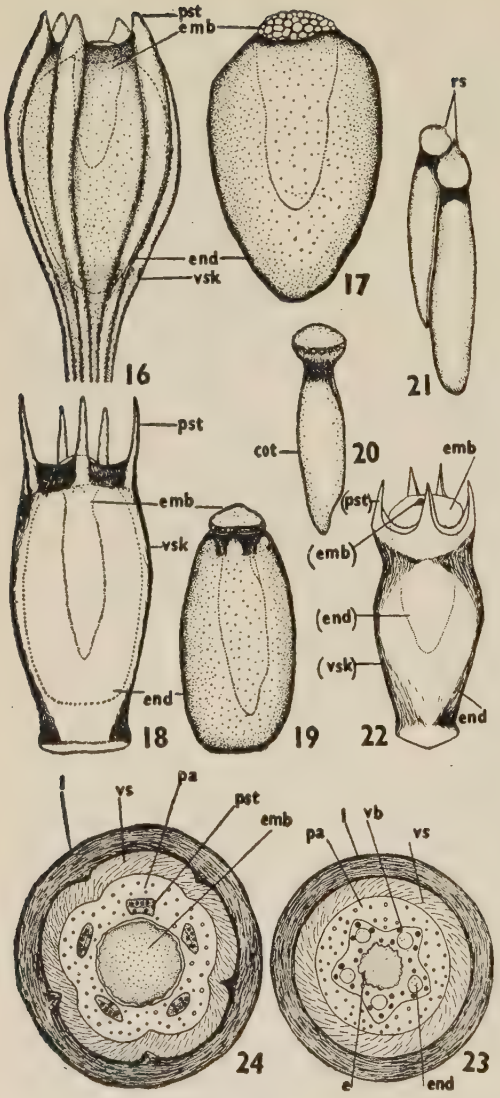
The ovoid fruit is a pseudo-berry with persistent calyculus. There is no seed-coat and the massive endosperm is directly surrounded by the pericarp. The latter is distinguishable into 4 zones. The outermost is tough and leathery and contains tannin filled cells and sclereides. The next is the viscid layer which is composed of thin-walled diagonally elongated cells. The third is a parenchymatous zone consisting of transversely elongated cells. The innermost zone contains the vascular bundles, shown in Fig. 16.

Dendrophthoe falcata

Singh's (1952) account of *Dendrophthoe falcata* is so complete that I am unable to add much to it. It appears, however, that some of his interpretations about the form and structure of the endosperm are not quite correct.

The mature endosperm has a massive flattened basal part which gradually broadens in the middle and narrows again at the apex (Figs. 18, 19). Whereas the base is solid, the upper part has a hollow cavity containing the embryo (Fig. 19), which differs from that of *D. neelgherrensis* in that the papillate outgrowths at the radicular end are situated only along its equatorial region and not on the entire surface (Fig. 20). Another feature of interest is the presence of twin embryos in many mature fruits (Fig. 21).

Singh (1952) states that the apical part of the endosperm is 5-pronged at the upper end. To quote (p. 468): " The shape of mature endosperm may be compared to a flower vase, with very massive walls and an even more massive basal part, which is narrow below, gradually widens towards the middle and narrows



FIGS. 16-24 — Figs. 16, 17 of *D. neelgherrensis* and 18-24 of *D. falcata* (*cot*, cotylendons; *e*, embryo; *emb*, embryo; *end*, endosperm; *l*, leathery coat; *pa*, parenchymatous zone; *pst*, remnants of perianth and staminal bundles; *rs*, remnants of suspensor; *vb*, vascular zone; *vs*, viscid layer; *vsk*, vascular skeleton). Fig. 16. Fruit in which the outer tissues have been removed to show the vascular skeleton enclosing the endosperm and embryo. × 7. Fig. 17. Older stage, in which vascular skeleton has also been removed; note endosperm and embryo. × 7. Figs. 18, 19. Similar to Figs. 16, 17. × 4. Fig. 20. Embryo. × 4. Fig. 21 Twin embryos. × 7. Fig. 22. Singh's (1952) Text-fig. 64 showing "perspective view of mature endosperm". Lettering in brackets shows present author's interpretation. × 12. Fig. 23. Singh's (1952) Text-fig. 62, to show T.S. fruit passing through upper part of endosperm and radicular end of embryo. The 5 circles marked *end* are interpreted by him as endosperm prongs. Fig. 24. T.S. fruit at about same level as Fig. 23. × 5.

again into a neck surmounted by the five sharply conical outgrowths (Text-fig. 64)". Further on (p. 469): "Close to the radicle end of the embryo the arrangement of the various tissues begins to show some changes. At this level (Pl. 23, Fig. 8, *V*) the endosperm is present only in five patches (*end*) which are the transversely cut ends of its conical prongs (Text-fig. 62 and Pl. 23, Fig. 13). Each of these patches is surrounded by the vascular zone (*vs*)." My observations show that although the upper margin of the endosperm, situated just below the projecting radicular end of the embryo, is somewhat wavy, there is no trace of any outgrowths or prongs. It appears instead that what Singh labels as embryo in his Text-fig. 64 (reproduced here as Fig. 22), is actually the endosperm with the embryo embedded inside it. The structure labelled as the endosperm would then be the vascular zone of the pericarp with the five prongs representing the vascular supply to the perianth and stamens. Transverse sections of the fruit at the level of the radicular end of the embryo do not show any trace of endosperm as the latter ends below this level (Fig. 24). The structures marked *end* in Singh's cross-section of the fruit (see his Text-fig. 62 reproduced here as Fig. 23) really belong to the pericarp.

Summary and Conclusion

The embryology of *D. neelgherrensis* and the endosperm and embryo of *D. falcata* are described.

In *D. neelgherrensis* a mass of subepidermal archesporium differentiates at the base of the ovarian cavity. Due to the elongation of the megaspore mother cells a conical projection, the "mamelon", is formed in the ovarian cavity. The meiotic divisions lead to linear tetrads of megaspores. The basal megaspores function and several embryo sacs, usually 7-10,

grow up to more than three-fourths of the length of the style. The first division of the zygote is vertical and is followed by many transverse divisions. Approximately half a dozen proembryos are seen in the style. Owing to the extreme elongation of the suspensor cells they are later pushed down into the ovary. A composite endosperm is formed by the fusion of endosperms of all the embryo sacs in the same ovary. Usually one embryo reaches maturity but sometimes two may be present. The radicular end of the pseudo-monocotyledonous embryo is covered with papillate processes. The fruit wall comprises four zones as in other Loranthoideae.

The apical part of the endosperm is devoid of the teeth or prongs reported by Singh.

I am highly indebted to Prof. P. Maheshwari and Dr. B. M. Johri for their guidance and help throughout the course of this work. To the Government of Mysore I am thankful for the award of a Scholarship.

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THE EMBRYOLOGY OF *IPHIGENIA INDICA* KUNTH.

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Introduction

A considerable amount of embryological work has been done on members of the family Liliaceae. These have been favourite objects for embryologists and cytologists because of the presence of large nuclei which stain readily and brightly.

Iphigenia indica belongs to the tribe Anguillarieae of the sub-family Melanthioideae (see Krause, 1930). Hutchinson (1934) divided the family Liliaceae into 28 tribes and assigned *Iphigenia*, *Ornithoglossum*, *Camptorrhiza*, *Reya* (*Burchardia*) and *Androcymbium* to the tribe Iphigeneae. *Iphigenia indica* has been partially studied by Joshi (1939). There is no work on any of the other genera. According to Joshi the archesporial cell cuts off a parietal cell. Usually the chalazal megaspore of the tetrad produces the embryo sac but the other three megaspores may also become two-nucleate. Further development conforms to the Polygonum type although occasionally, bisporic development (Allium type) may also occur. The mature embryo sac shows multinucleate antipodal cells.

Material and Methods

Fixed material of *Iphigenia indica* was very kindly collected and sent by Dr. K. R. Venkatasubban (Madras), Mr. T. Thathachar (Bangalore), Dr. B. G. L. Swamy (Madras), Mr. J. V. Deshpande (Bombay), Mr. H. R. Bhargava (Sagar) and Miss A. T. Zachariah (Madras). Some embedded material and prepared slides were kindly passed on to me by Prof. P. Maheshwari and Dr. B. M. Johri. To all of them I am most grateful.

Fixations were made in formalin-acetic-alcohol (formalin 5 c.c., glacial acetic acid 5 c.c., and 50 per cent ethyl alcohol 90 c.c.). Mature seeds were softened with 10 per cent hydrofluoric acid (diluted with 70 per cent alcohol) for ten days. The usual methods of dehydration and embedding were followed. Sections were cut 7-16 μ thick and stained in safranin-fast green as well as Heidenhain's iron-haematoxylin. The latter combination gave better results. Anther smears were stained with a mixture of equal volumes of 50 per cent glycerine and acetocarmine.

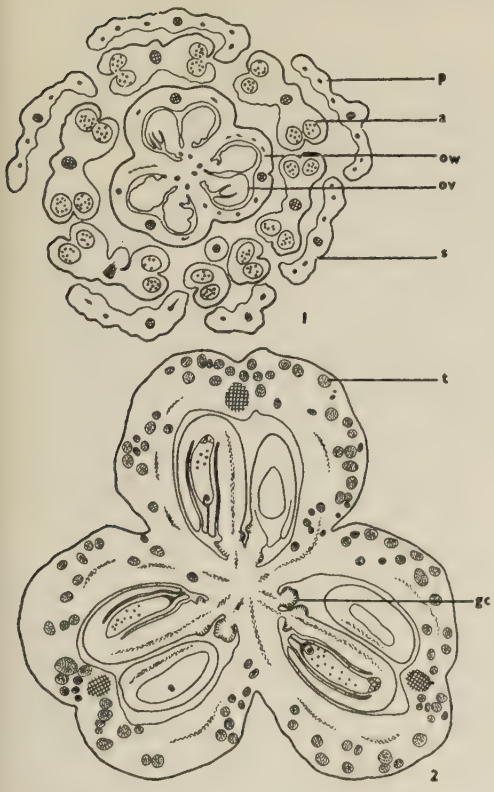
Observations

Flower and Fruit — The small bracteate purplish flowers are borne in short racemes. The segments of the six-partite deciduous perianth spread out in a stellate fashion. There are six hypogynous stamens with oblong, versatile anthers. The trilocular, superior ovary has a short style bearing a trifid stigma. Two rows of anatropous ovules occur in each locule on axile placentae (Fig. 2). Fig. 1 is a cross-section of the flower showing the disposition of the various parts.

Some ovaries showed axile placentation in the upper and lower parts and parietal in the middle part, as the carpellary margins had not fused in this region.

At the mature embryo sac stage the epidermal cells at the base of the funiculus become richly protoplasmic and glandular (Figs. 2, 20 and 32). The ovary wall is about 12-13 cells thick and many of the cells become prominent due to the deposition of tannin (Fig. 2).

The fruit is a three-valved loculicidal capsule containing 10-13 subglobose seeds in each of the six rows. The seeds have a hard brown testa, copious endosperm and a comparatively small monocotyledonous embryo.



FIGS. 1, 2 — Fig. 1. T.S. open flower (*a*, anther; *ov*, ovule; *ow*, ovary wall; *p*, perianth; *s*, stomata). × 20. Fig. 2. T.S. ovary, post-fertilization (*gc*, glandular cells; *t*, tannin-filled cell). × 30.

MICROSPORANGIUM — The four-lobed anther shows an elongated sporangium in each lobe. At maturity the two adjacent pollen sacs become confluent due to the breaking down of the partition between them (Figs. 3, 4). The archesporial cells divide to form the primary parietal layer and the primary sporogenous tissue. The former divides again giving rise to the endothecium and an inner layer which undergoes several divisions resulting in 2-3 middle layers and the glandular tapetum. Thus the wall of the anther comprises 5-6 layers including the epidermis.

During reduction divisions the epidermal cells become papillate and vacuolated. The radially elongated cells of the endothecium develop fibrous thickenings, but the cells situated in the region of dehiscence (Figs. 3, 4) remain small and thin-walled. Next to the endothecium are 2-3 middle layers. The innermost is crushed during meiotic divisions of the microspore mother cells, while the outermost or the outer two persist in the mature anther. Many cells of the connective and occasionally some adjacent cells of the middle layer also develop fibrous thickenings (Fig. 4).

The large densely cytoplasmic tapetal cells are originally uninucleate, but at the time of reduction divisions some of them become binucleate (Fig. 5).

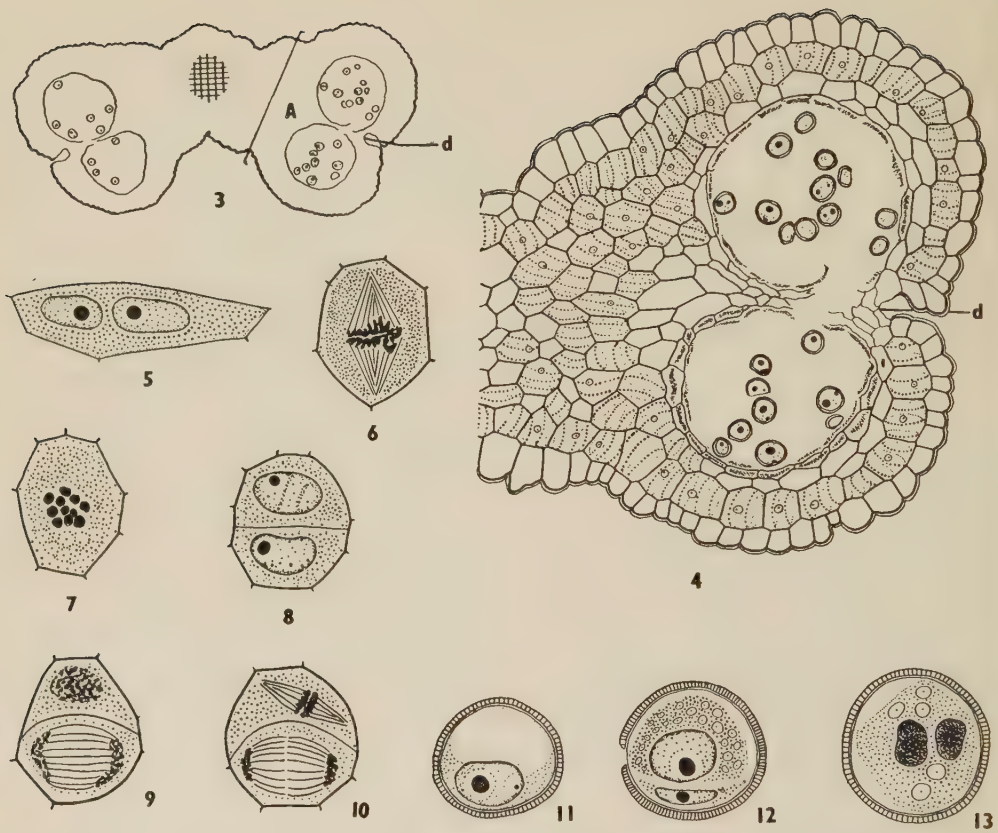
The primary sporogenous cells undergo a few mitotic divisions to give rise to the microspore mother cells. The reduction divisions are successive (Figs. 6, 8) and cytokinesis is by cell plate formation. Both isobilateral and tetrahedral tetrads occur (Figs. 9, 10), but the former are more common. During meiosis a special mucilaginous layer is secreted between the original wall of the mother cell and its protoplast. This is consumed during the separation of the microspores from the tetrads.

In equatorial views of metaphase plates the microspore mother cells showed 11 pairs of chromosomes (Fig. 7). Joshi (1939) has also recorded the same number.

In the same flower different anthers may show all stages of development from microspore mother cells to uninucleate pollen grains.

MALE GAMETOPHYTE — The newly formed microspores have dense cytoplasm. As they increase in size, a central vacuole appears which displaces the nucleus towards the wall (Fig. 11). The pollen grain has a single germinal furrow (Fig. 12). The microspore nucleus divides to give rise to a larger vegetative and a smaller lenticular generative cell. The pollen grain contains abundant starch and is shed at the 2-celled stage (Fig. 12).

One abnormal pollen grain showed two nuclei of approximately equal size (Fig. 13). Such a condition may originate due to failure of wall formation during the second reduction division; or, the first division of the microspore nucleus may sometimes give rise to two daughter nuclei of similar size.

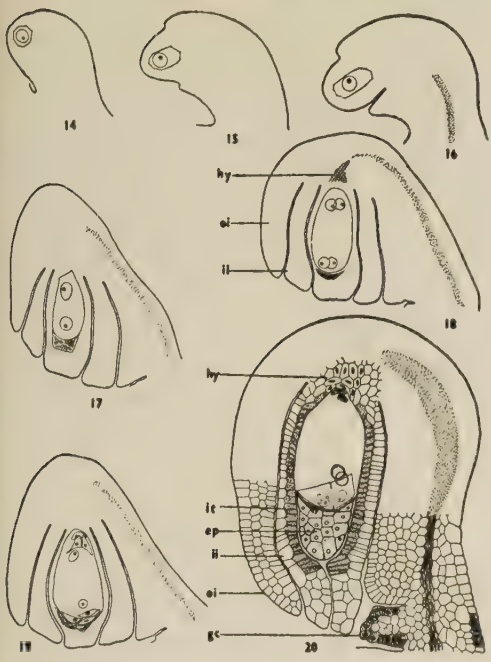


FIGS. 3-13 — Fig. 3. T.S. mature anther, diagrammatic (*d*, region of dehiscence). × 72. Fig. 4. Part of the anther marked A in Fig. 3, enlarged to show the region of dehiscence, *d*; many cells of the connective have also developed fibrous thickenings. × 200. Fig. 5. Two-nucleate tapetal cell. × 1175. Fig. 6. Microspore mother cell, Meiosis I. × 1175. Fig. 7. Same, 11 pairs of chromosomes are seen in equatorial view. × 1175. Fig. 8. Dyad. × 1175. Figs. 9, 10. Meiosis II. × 1175. Figs. 11, 12. Uni- and bicelled pollen grains. × 1175. Fig. 13. Abnormal pollen grain. × 1175.

OVULE — The ovule is initiated as a small protuberance. The inner integument and the archesporial cell differentiate at about the same time[1]. By the time the reduction divisions are over, both the integuments are considerably advanced. Due to rapid growth on one side the ovule becomes anatropous (Figs. 14-20). The inner integument is 2- to 3-layered and the outer one 3- to 5-layered (Fig. 20). The micropyle is formed by the inner integument only.

The nucellus is poorly developed. At the 4-nucleate stage some of the chalazal cells, between the embryo sac and the funicular vascular supply, become prominent and densely cytoplasmic. These constitute the "hypostase". Its cells remain thin-walled throughout (Figs. 20, 32) and are consumed during post-fertilization stages as also in *Yucca filamentosa*

1. In this connection Joshi (1939) states: "The archesporium differentiates as the carpellary margins meet one another. The inner integument has appeared by this time above and below but not on the sides of the nucellus. This is clear from the fact that its initials are visible in longitudinal sections of the ovary (Fig. 11) but not in transverse sections (Fig. 12)." I have not been able to understand the latter part of this statement which is confused and unintelligible. Joshi's Figs. 11 and 12 are reproduced here as Figs. 62 and 63 respectively.



FIGS. 14-20 — Figs. 14-16. L.S. ovules at megaspore mother cell stage. × 160. Figs. 17, 18. Same, at 2- and 4-nucleate embryo sac stages (*hy*, hypostase; *ii*, inner integument; *oi*, outer integument). × 160. Figs. 19, 20. L.S. ovules at mature embryo sac stage (*ep*, epistase, *gc*, glandular cells; *hy*, hypostase; *ii*, inner integument; *it*, integumentary tapetum; *oi*, outer integument). × 160.

(Wunderlich, 1938), *Gloriosa superba* (Joshi, 1939), and *Gagea fascicularis* (Joshi, 1939).

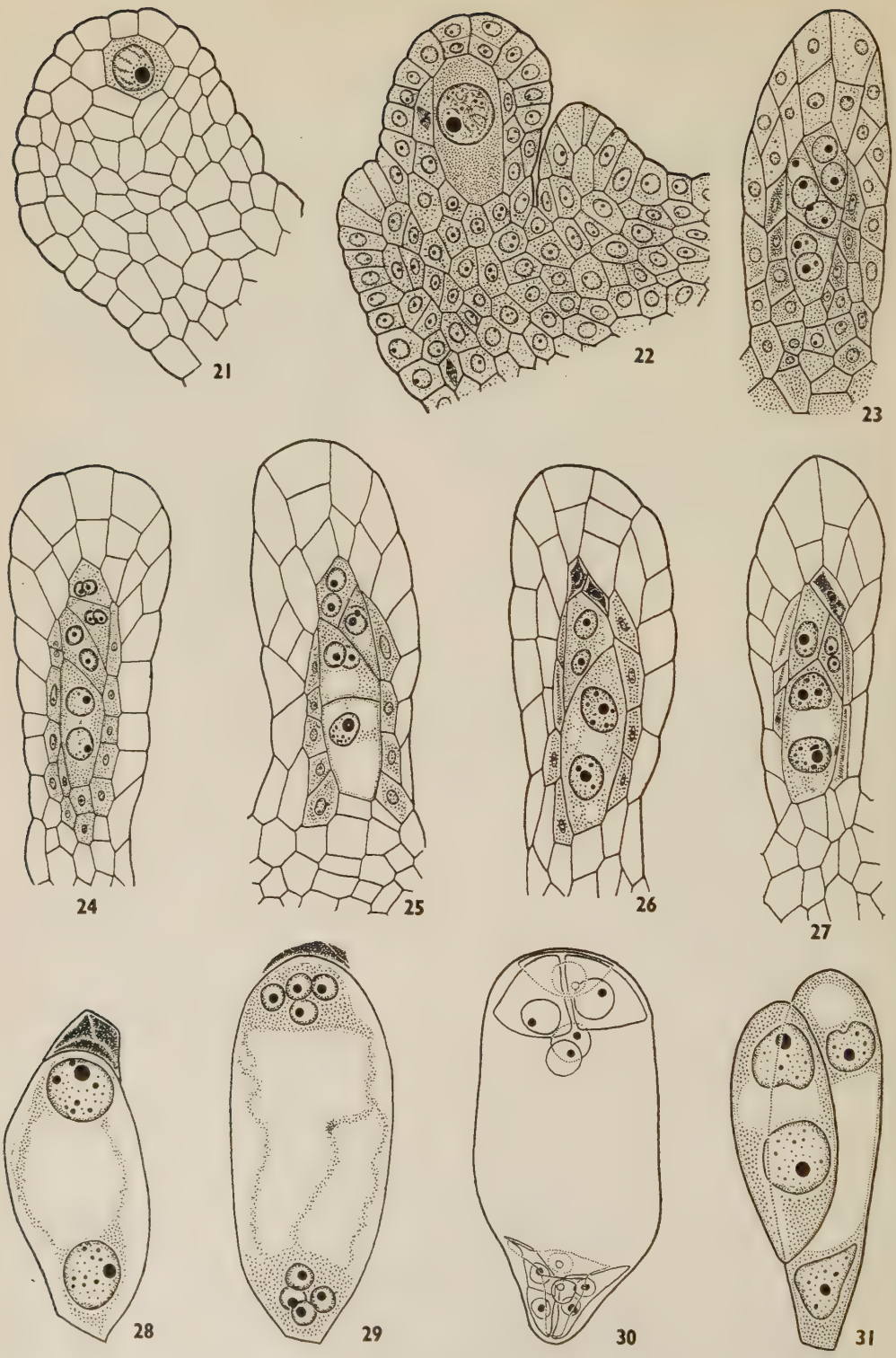
The nucellar epidermis undergoes periclinal divisions (Figs. 22-27) resulting in the formation of an epidermal cap which forms the epistase. At the mature embryo sac stage its cells show larger nuclei and a highly vacuolated cytoplasm (Fig. 20). Later the walls become cutinized and it persists until the globular stage of the embryo (Fig. 39). Eventually it is crushed and consumed and there is no trace of it in the seed. A similar epistase has also been recorded in *Gloriosa* (Joshi, 1939) and *Albuca* (Eunus, 1950).

Due to a lateral expansion of the embryo sac the nucellar cells are crushed so that the former comes to lie in direct contact with the inner epidermis of the inner integument which differentiates into an endothelium (Figs. 20, 32).

MEGASPOROGENESIS — A single hypodermal archesporial cell differentiates in the young nucellus (Fig. 21), but sometimes there may be a multicelled condition. The archesporial cell directly functions as the megaspore mother cell. Joshi (1939) states: "The functional archesporial cell invariably cuts off a parietal cell, but this or its daughter cells never divide periclinally so that only one layer of parietal cells is formed (Figs. 7, 13 and 22)." I have never found a parietal cell (Fig. 22) and consider Joshi's interpretation to be incorrect. What he designates as parietal cells seem really to be the derivatives of the nucellar epidermis (see also Joshi's own Figs. 7, 13 and 22 reproduced here as Figs. 60, 61 and 62 respectively).

Joshi (1939) makes yet another erroneous interpretation when he speaks of a nucellar tapetum surrounding the megaspore mother cell (see his Fig. 13 reproduced here as Fig. 61). As shown in Fig. 22, at this stage all the nucellar cells are characterized by large nuclei and dense cytoplasm. It is at a later stage, during the meiotic divisions of the megaspore mother cell, that the derivatives of the nucellar epidermis become vacuolated and thus offer sharp contrast with the more inwardly situated cells which still retain their dense cytoplasm and remain unchanged for some time longer. It is these cells which have been designated by Joshi as tapetal. However, at no time do they show any increased metabolic activity such as is characteristic of a real tapetum and soon degenerate and disappear (Figs. 25-27).

The megaspore mother cell undergoes the usual reduction divisions to form a tetrad of megaspores. Fig. 24 shows a linear tetrad all the megaspores of which are in binucleate condition. Fig. 25 shows a T-shaped tetrad in which the chalazal megaspore has considerably enlarged and seems destined to develop into the embryo sac. However, each of the other three megaspores is already binucleate. Fig. 26 shows another T-shaped tetrad in which the upper two megaspores have degenerated while the lower two have developed to the 2-nucleate stage.



FIGS. 21-31.

The condition in Fig. 27 is more difficult to interpret. Here we have a degenerating megaspore at the micropylar end, then 2 megaspores (the right one binucleate) lying parallel to one another, and finally a large binucleate chalazal cell. Hjelmqvist (1951) has reported an arrangement of megaspores in *Tridax trilobata* which seems to offer some resemblance. There is no wall formation after Meiosis I. After Meiosis II the walls are laid down in such a way as to form three cells of which the middle one is binucleate while the other two are uninucleate. Since the embryo sac arises from the middle cell, it is bisporic. However, if a vertical wall were laid down between the two nuclei of the middle cell, it would result in a condition described above in *Iphigenia*.

Female Gametophyte — An advanced 2-nucleate embryo sac shows the nuclei separated by a large vacuole (Fig. 28). By this time the 3 non-functional megaspores have degenerated and appear as darkly stained masses above the embryo sac. The 4- and 8-nucleate (Fig. 29) embryo sacs are formed in the usual way. Fig. 30 shows an organized embryo sac. The synergids lack the characteristic basal vacuoles. Ordinarily they are short-lived structures but may sometimes persist even after fertilization. In one case a small proembryo appears to have developed from one of the synergids (Fig. 53). The polar nuclei fuse in the micropylar half of the embryo sac. At first the antipodal cells are small and uninucleate but later on they show an increase both in size and number and persist until a late stage (Figs. 33-35). There is also an increase in the number of nuclei in each antipodal cell. Subsequently nuclear fusions occur in groups of 3-4 resulting in large polyploid nuclei having an irregular outline.

Fig. 31 shows a case of twin embryo sacs. It is difficult to say whether they originated from two megaspores of the same tetrad or of two different tetrads.

Endosperm — The shape and size of the embryo sac changes considerably after fertilization (Figs. 32-35). Its chalazal end elongates and curves to form a pouch which contains the large multinucleate antipodal cells (Figs. 34, 35).

The endosperm is Nuclear. The division of the primary endosperm nucleus precedes that of the zygote (Fig. 32). Many endosperm nuclei are formed, which are at first distributed throughout the embryo sac. In later stages, they take up a peripheral position and some of them aggregate at the chalazal end of the embryo sac. Wall formation is centripetal and by the time the proembryo is globular the whole of the endosperm is cellular (Figs. 38, 39).

In one ovule with an 8-celled proembryo many of the endosperm cells were multinucleate and the nuclei were in a process of fusion resulting in the formation of some large polyploid nuclei with lobed outlines (Figs. 36, 37). I am unable to say if this is a regular feature for in older stages the cells were generally uninucleate (Fig. 39).

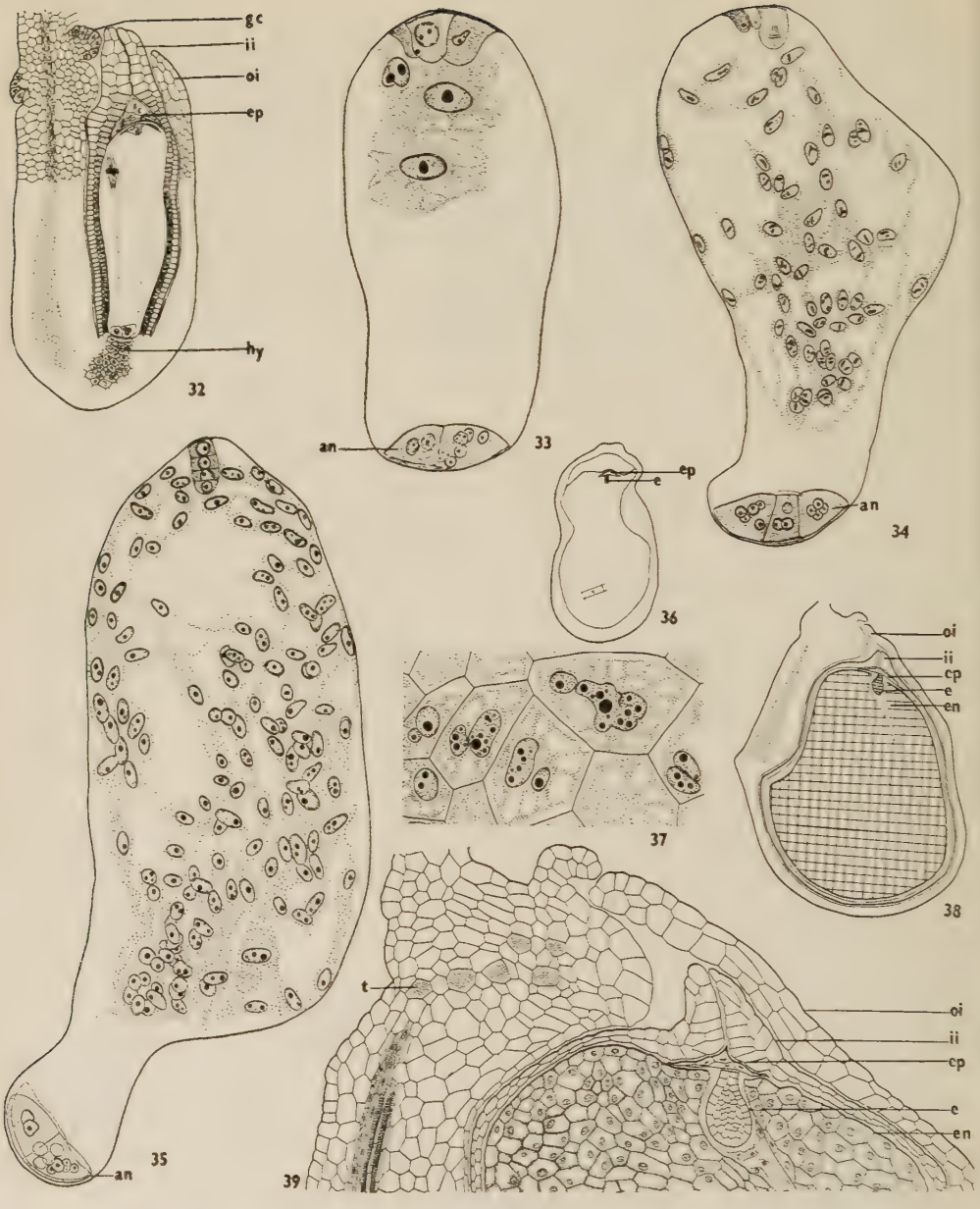
Embryo — The zygote enlarges and shows vacuolated cytoplasm and a prominent nucleus (Fig. 40). It divides only after about 60 free endosperm nuclei have been formed (Fig. 34). The first division is transverse and gives rise to a terminal and a basal cell (Fig. 41). A 4-celled proembryo may be formed in two ways:

(1) The basal as well as terminal cells may both divide transversely resulting in a 4-celled filamentous proembryo (Figs. 42, 43).

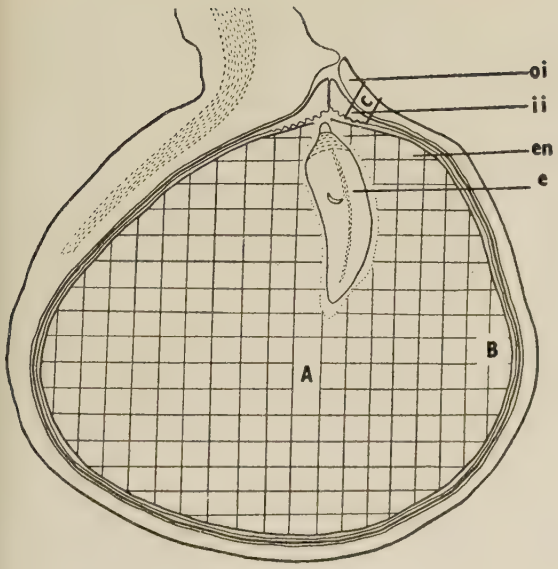
(2) The terminal cell may divide vertically while the basal cell divides transversely resulting in a ⊥-shaped proembryo (Figs. 44, 45).

←

Figs. 21-31 — Figs. 21, 22. Hypodermal archesporial cell and megaspore mother cell respectively. × 450. Fig. 23. L.S. nucellus at megaspore tetrad stage; cells immediately surrounding the tetrad have dense cytoplasm as compared to the outer cells of the nucellus which are highly vacuolated; all the four megaspores are binucleate. × 450. Figs. 24-27. Tetrads showing various dispositions of megaspores; explanation in text. × 450. Figs. 28, 29. 2- and 8-nucleate embryo sacs. × 450. Fig. 30. Mature embryo sac; of the five antipodal cells one is binucleate. × 450. Fig. 1. Two-nucleate twin embryo sacs. × 450.



FIGS. 32-39 — Fig. 32. L.S. ovule; embryo sac shows the first division of the primary endosperm nucleus (*ep*, epistase; *gc*, glandular cells; *hy*, hypostase; *ii*, inner integument; *oi*, outer integument). × 113. Figs. 33-35. Free nuclear endosperm; the pouch-like chalazal end of the embryo sac (in Figs. 34 and 35) contains multinucleate antipodal cells (*an*). Fig. 33. × 273. Figs. 34 and 35. × 212. Fig. 36. L.S. ovule at 8-celled proembryo stage (*e*, proembryo; *ep*, epistase). × 26. Fig. 37. Portion of cellular endosperm, marked X in Fig. 36, enlarged to show nuclear fusions. × 439. Fig. 38. L.S. ovule at globular proembryo stage (*e*, proembryo; *en*, endosperm; *ep*, epistase; *ii*, inner integument; *oi*, outer integument). × 27. Fig. 39. Same, upper portion enlarged to show the integuments, epistase, proembryo and part of the cellular endosperm (*t*, tannin-filled cell). × 113.



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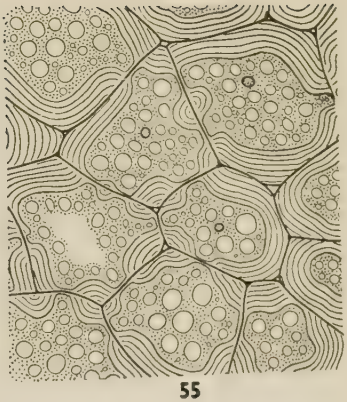

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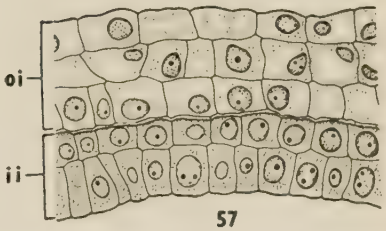

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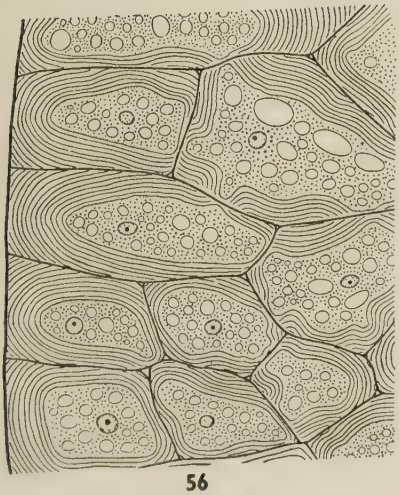

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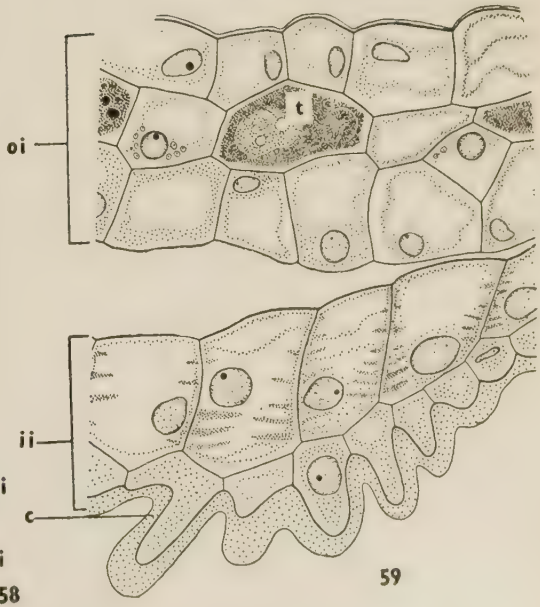

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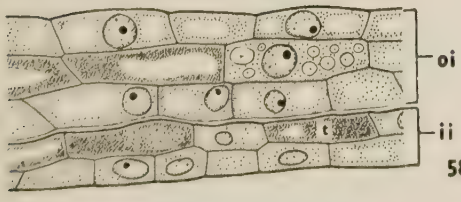

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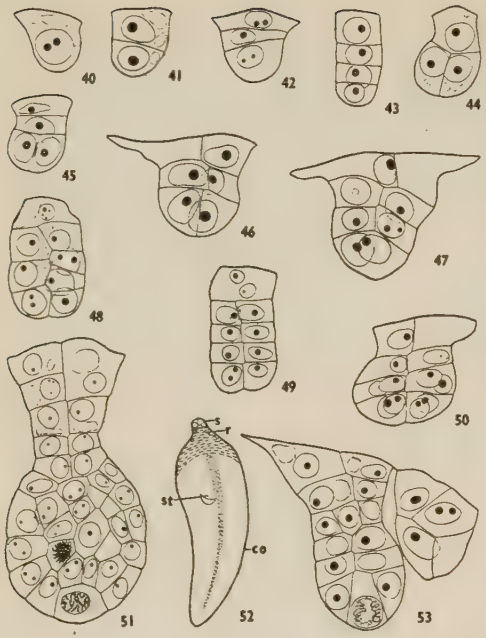
FIGS. 54-59 — Fig. 54. L.S. mature seed (*e*, embryo; *en*, endosperm; *ii*, inner integument; *oi*, outer integument). × 31. Figs. 55, 56. Portions of endosperm from places marked A and B respectively in Fig. 54, enlarged to show detailed structure. × 310 Figs. 57-59. Development of testa; Fig. 59 presents an enlarged view of portion marked C in Fig. 54 (*c*, cuticle; *ii*, inner integument; *oi*, outer integument; *t*, tannin). × 500.



FIGS. 40-53 — Fig. 40. Zygote. × 363. Figs. 41-51. Development of proembryo; explanation in text. × 363. Fig. 52. Mature embryo (*co*, cotyledon; *r*, root cap; *s*, suspensor; *st*, stem tip). × 36. Fig. 53. Twin embryos, one probably derived from a synergid. × 363.

In either type of proembryo transverse as well as longitudinal divisions take place and lead to the formation of 3-5 biseriate tiers (Figs. 46-50). The two terminal tiers divide repeatedly and produce a globular mass of cells (Fig. 51). Ultimately there develops an embryo with a single cotyledon, a lateral stem tip and a small 5- to 6-celled suspensor (Fig. 52).

Fig. 53 shows twin embryos, the one on the left being zygotic and the other probably derived from the persistent synergid.

At the mature embryo sac stage the two layers of the inner integument are densely protoplasmic while the three layers of cells comprising the outer integument are highly vacuolated. The integuments do not show any appreciable change during early stages of embryogeny. At the globular stage of the embryo, the outer integument enlarges and extends upward so that the inner integument is completely covered (Figs. 38, 39, 54).

Both the integuments take part in the formation of the seed coat (Figs. 57-59). The outer epidermis of the outer integument develops a thin cuticle. At the same time the inner epidermis of the inner integument becomes heavily cutinized while the outer develops tangential thickenings (Figs. 54, 59). At this stage the two layers of the inner integument have a markedly different staining capacity.

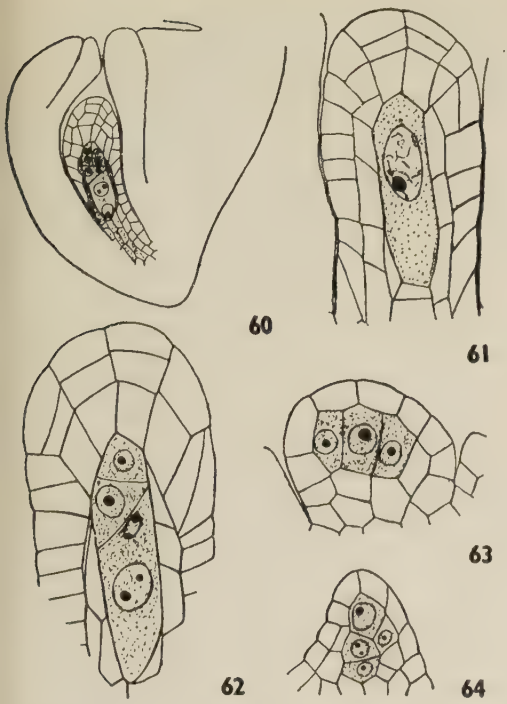
Due to a deposition of hemicellulose, the walls of the endosperm cells become greatly thickened and pitted. Figs. 55 and 56 are enlarged views of portions from the central and peripheral regions respectively of the endosperm at the stage shown in Fig. 54. The peripheral cells show a greater thickening and have fewer pits as compared to those in the central region. Hemicellulose seems to be the chief food reserve. Oil is also present in the cells.

The cells of the funiculus and of the middle layer of the outer integument contain abundant starch. Some of the cells also contain tannin (Figs. 39, 59). The mature seed is moderately hard, dark brown in colour, and includes a copious endosperm enclosing a small monocotyledonous embryo.

Discussion

Joshi (1939) writes: "A tapetum differentiates during the development of the embryo sac. It first arises from the cells of the nucellus surrounding the megaspore mother cell (Fig. 13)." As shown earlier Joshi misinterpreted the disorganizing nucellar cells as of tapetal nature. His Fig 13, reproduced here as Fig. 61, does not show any tapetal tissue.

With regard to the parietal tissue two conditions occur in the Liliaceae. The hypodermal archesporial cell may divide to form a primary parietal and a primary sporogenous cell as in the Scilloideae, or it may function directly as a megaspore mother cell as in the Lilioideae. Joshi (1939) reports a wall cell in *Iphigenia indica*. This, however, seems to be incorrect as already shown on page 183. In some plants like *Ophiopogon wallichianus* (Maheshwari, 1934) the condition is



FIGS. 60-64 — Copies of Joshi's Figs. 7, 13, 22, 11 and 12 respectively. Explanation in text. × 143, 400, 393, 424, 420.

variable[2] and a wall cell may or may not be cut off so that the megaspore mother cell may be situated directly below the nucellar epidermis, or it may be separated from the latter by a parietal cell. In *Urginea indica* (Capoor, 1937) the primary parietal cell, sometimes, assumes a sporogenous function so that two megaspore tetrads are formed in the same row. It appears that Joshi's wall cells are really derivatives of the nucellar epidermis.

Of interest in this connection is also the recent report of Gorham (1953) on the archesporial cell of *Smilacina racemosa*. She states that " It is situated below one hypodermal nucellar layer which usually is two cells wide. The archesporial cell functions directly as the megaspore mother cell (Fig. 2). Division in the hypodermal layer occurs first in one cell, then the other; thus in sections, the megaspore mother cell may appear to be separated from the nucellar epidermis by one or two parietal layers (Figs. 2, 3). In later stages, there are two parietal layers between the embryo sac and the nucellar epidermis." Gorham seems to think that the archesporial cell is differentiated in the third layer of the nucellus. Her Fig. 1 clearly shows, however, that the megaspore mother cell is separated from the epidermis by a primary parietal cell. In my opinion she has erroneously interpreted the former as the archesporial cell and the latter as a ' hypodermal nucellar cell '. Maheshwari's (1950) generalized statement that the archesporial cell is of hypodermal origin and that reports of its origin from the third layer of cells in the nucellus are doubtful, is therefore quite satisfactory so far as present records are concerned.

Five types of embryo sac development are known in the Liliaceae. In the monosporic types usually the chalazal megaspore functions but occasionally the other megaspores may also develop up to the 2-nucleate stage as in *Galtonia*, *Uvularia* (see Schnarf, 1931) and *Gloriosa* (Eunus, 1949). *Iphigenia indica* belongs to the same category. Joshi (1939) has recorded two exceptional cases. One is interpreted as showing Allium type of development and the other as intermediate between the Polygonum type and the Allium type. He writes: " Three cases were seen by me in which it appears that no wall had appeared in the chalazal dyad cell after the second meiotic division and the nuclei formed as a result of this had been equal in size, so that the chalazal dyad cell had directly given rise to a 2-nucleate embryo-sac (Fig. 23). These embryo-sacs differed from other 2-nucleate embryo-sacs of the same size in the absence of any signs of vacuolation and it seems certain that the 8-nucleate embryo-sac in these cases would have developed according to the *Scilla*-type." As regards the intermediate type, he says: " In Fig. 22 no wall has appeared in the chalazal dyad cell after the second meiotic division.

2. Dahlgren (1927) has given a table enumerating those plants of the family in which wall cells are present and others in which they are absent. In *Hemerocallis fulva*, *Polygonatum commutatum*, *Medeola virgianica*, *Paris quadrifolia*, *Smilacina amplexicaulis*, *Smilacina racemosa*, and *Smilacina stellata*, wall cells are present in some ovules and absent in others.

Also the nucleus formed towards the micropylar side is small and likely to degenerate during further development. The embryo-sac in this case would have developed from the cytoplasm of two but nucleus of one megaspore. It would thus be intermediate between the *Normal-* and the *Scilla*-types."

While my material showed tetrads of various shapes, I did not come across any such cases and the development always followed the monosporic plan.

In the organization of the mature embryo sac an important feature is the persistence of one synergid. This has also been recorded in some other genera of the Liliaceae such as *Allium* (Weber, 1929), *Nothoscordum* (Stenar, 1932), *Muscari* (Wunderlich, 1937) and *Albuca* (Eunus, 1950). In *Iphigenia indica* one of the synergids may even develop into an embryo. Synergid embryos have also been reported in *Allium rotundum* and *A. zebdanense* (Weber, 1929).

The antipodals show a great deal of variation in the Liliaceae. In several genera they are quite ephemeral. In *Scilla, Tricyrtis* and *Ornithogalum* (Schnarf, 1931) they are conspicuous and persistent and each shows a large hypertrophied nucleus. In *Gloriosa superba* the antipodal cells become binucleate (Eunus, 1949) Multinucleate persistent antipodals occur in *Heloniopsis zygadenus* (see Schnarf, 1931) and *Iphigenia*. In *Iphigenia* the nuclei in each antipodal cell fuse in groups of 3-4 resulting in large irregular polyploid masses. Another interesting feature is that the number of antipodal cells may increase up to five. However, no antipodal embryos were seen in this plant as recorded in *Allium odorum* (Modilewski, 1931; Håkansson, 1951).

Affinities — Since no work has been done on any other member of the Iphigenieae except *Iphigenia*, not much can be said as regards the affinities of this tribe. However, it shows some points of resemblance with the tribe Uvularieae. Common features between *Iphigenia* (Iphigenieae) and *Gloriosa* (Uvularieae) are: (1) anatropous bitegmic ovules; (2) absence of parietal cells in nucellus; (3) presence of hypostase; (4) well-developed nucellar cap; (5) development of non-functional megaspores up to 2-nucleate stage; (6) monosporic 8-nucleate embryo sac; (7) multinucleate antipodal cells; and (8) Nuclear endosperm.

While further work on both the tribes is essential, present evidence seems to suggest a close affinity between the Iphigenieae and Uvularieae.

Summary

The flowers are trimerous and hypogynous and the placentation is axile. Occasionally the middle region of the ovary may show parietal placentation due to incomplete fusion of the septa.

The anther wall comprises the epidermis, fibrous endothecium, 2-3 middle layers and a multinucleate glandular tapetum. At the time of dehiscence, one or two middle layers may persist. The reduction divisions are of the successive type resulting in tetrahedral or isobilateral tetrads. The haploid number of chromosomes is eleven.

The pollen grains are shed at the 2-celled stage and contain abundant starch.

The ovules are anatropous and bitegmic. At first the micropyle is formed by the inner integument only, but in post fertilization stages the outer integument surpasses the inner so that an exostome is also present. The slender nucellus becomes consumed on the sides due to the expansion of the embryo sac. At maturity the latter comes to lie adjacent to the inner epidermis of the inner integument which differentiates into an endothelium. At the micropylar end the nucellar epidermis divides to form an epistase which persists till the globular stage of the embryo.

The hypodermal archesporial cell functions directly as the megaspore mother cell. No parietal cell is formed. The tetrads of megaspores may be linear or T-shaped. The chalazal megaspore functions but the upper three megaspores may frequently develop up to the 2-nucleate stage. The development of the embryo sac conforms to the Polygonum type.

The mature embryo sac shows the usual organization. Sometimes one of the synergids may persist even after fertilization. The antipodal cells increase in size and number and also become multinucleate.

Due to nuclear fusions in groups of 3-4, irregular polyploid nuclei are formed in each antipodal cell.

The endosperm is Nuclear. In one case twin proembryos were observed. The additional embryo seems to have originated from the persistent synergid.

Both the integuments take part in the formation of testa. The dark brown seed contains copious endosperm and a small monocotyledonous embryo. The endosperm cells store fat and their walls are highly thickened due to the deposition of hemicellulose.

It is a great pleasure to thank Prof. P. Maheshwari and Dr. B. M. Johri for their encouragement and guidance throughout the work.

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THE GAMETOPHYTE OF *CHEIROPLEURIA BICUSPIS* (BL.) PRESL.

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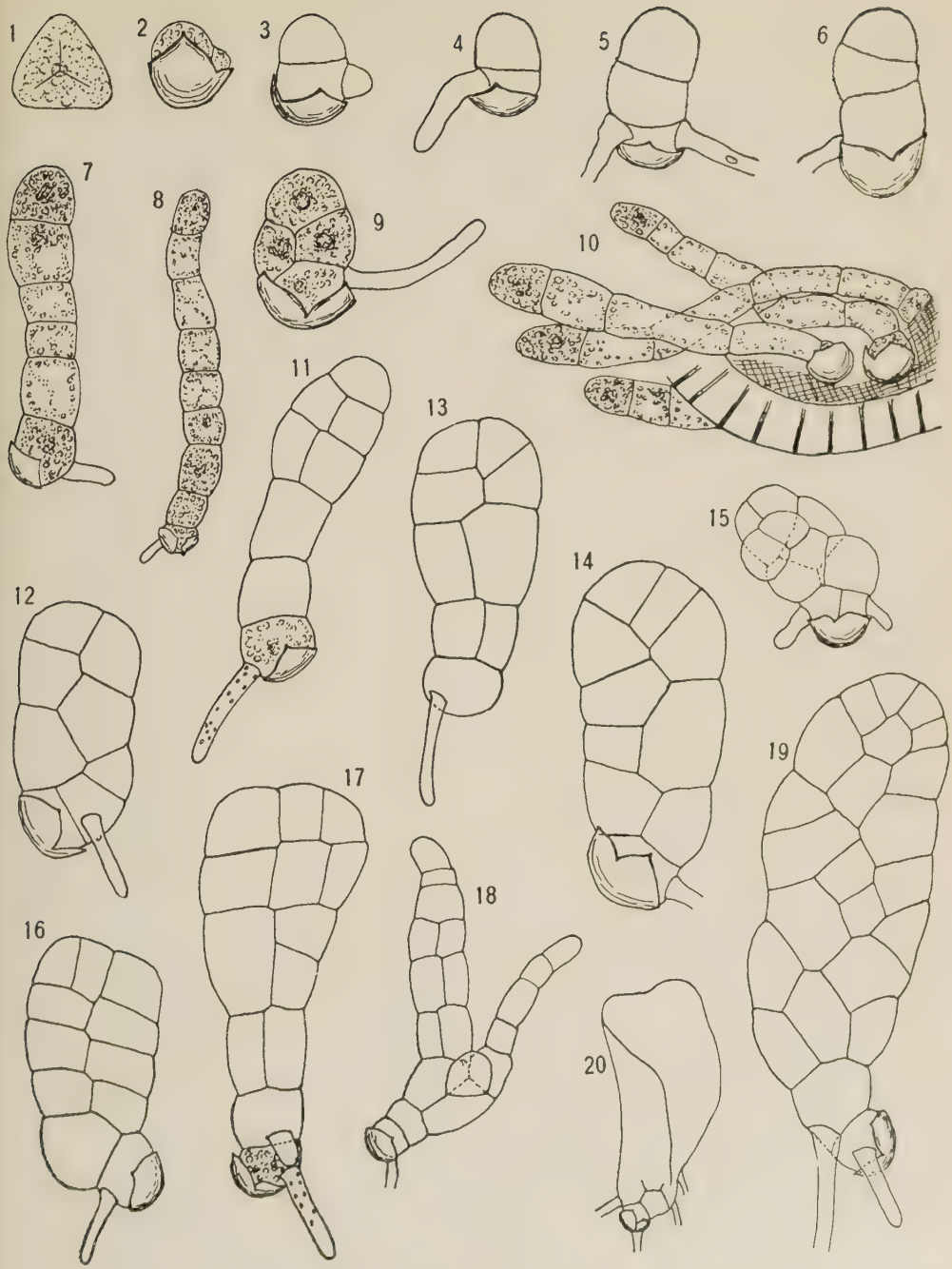
In 1928 Nakai proposed a new family, Cheiropleuriaceae, for the monotypic genus *Cheiropleuria*. In 1933 he described the gametophyte of *C. bicuspis* (Bl.) Presl. from material collected in a shady forest with mosses and other ferns. Although the material was not abundant and gave few stages, Nakai stated that the characters of the gametophyte as well as those of the sporophyte justified the elimination of *Cheiropleuria* from the Polypodiaceae. In view of the meagreness of his account and certain ambiguities it seemed desirable to have a more extended study of the gametophyte which would include early stages and a developmental series. We were fortunate to be able to obtain a good collection of spores from the Island of Okinawa through the kindness of Dr. E. H. Walker of the U.S. National Herbarium, and Mr. Tetsua Amano of the University of the Ryukyus.

The spores arrived by air mail on October 7, 1951, and were planted immediately, some on distilled water, some on porous clay crock lying on peat or *Sphagnum*, and some on peat. The spores have a good period of viability when kept under refrigeration; spores 18 months old germinated almost as readily as when received, but germination at two years was slower.

The spore of *C. bicuspis* is relatively small, with an average diameter of 35 μ; it is tetrahedral with a tripartite ridge visible but inconspicuous (Fig. 1). The individual spores are colourless but appear whitish in mass; the granular contents are plainly visible through the transparent coat. The spores tend to adhere to each other, and when planted they stick together so that many germinate in clumps of greater or less size, and relatively few germinate singly. Many remain in the ruptured sporangium and germinate there (Fig. 10). Germination was slow on all media although the percentage of germination was high. It was first seen at 16 days on water, and in 29 days on crock (Fig. 2); it was not until three or four weeks after planting that prothalli with two cells and a rhizoid were found on water (Figs. 3, 4). Growth continued to be slow, and seven weeks after planting most gametophytes had only 4-6 green cells and one or two rhizoids (Figs. 5, 6, 9).

Under favourable conditions germination was usually of a primitive type, although the prothalli showed greater plasticity and greater response to different light conditions than may be found in many primitive ferns. In the early stages there is usually a short filament as is indicated by Figs. 11, 17, or, less frequently, a plate (Fig. 9). The short filament of 3 or 4 cells may broaden immediately (Figs. 13, 14) leaving only one undivided cell at the base (Figs. 13, 14, 16, 20). The filament may develop to greater length but we rarely found as many as six cells except under very unfavourable conditions (Figs. 7, 8). The spore coat adheres persistently to the basal cell, and for weeks or even months the three valves may be found partly enveloping the basal cell; it is not readily displaced when material is mounted in water. The first prothallial cell, which is short even when the later cells are long, has a good complement of chloroplasts. They are present also in the first rhizoid and even in later rhizoids (Figs. 11, 17). Many gametophytes are so short and heavy that they suggest mass development, but no real case of mass development was observed, although thickening of the thallus may occur at an early



FIGS. 1-20 — Fig. 1. Spore. × 350. Figs. 2-6. Early stages of filamentous thallus. × 225. Figs. 7, 8, 18. Thalli from crowded clumps, 2-3 months. Fig. 9. Plate stage, 50 days. Fig. 10. Germination of spores in sporangium. Figs. 11-14, 16. Young plate stage, 2 months. × 215. Fig. 15. Thallus with division in third plane. Figs. 17, 19. Prothalli, 3 months. Fig. 20. Prothallus with uplifted wings, 3 months.

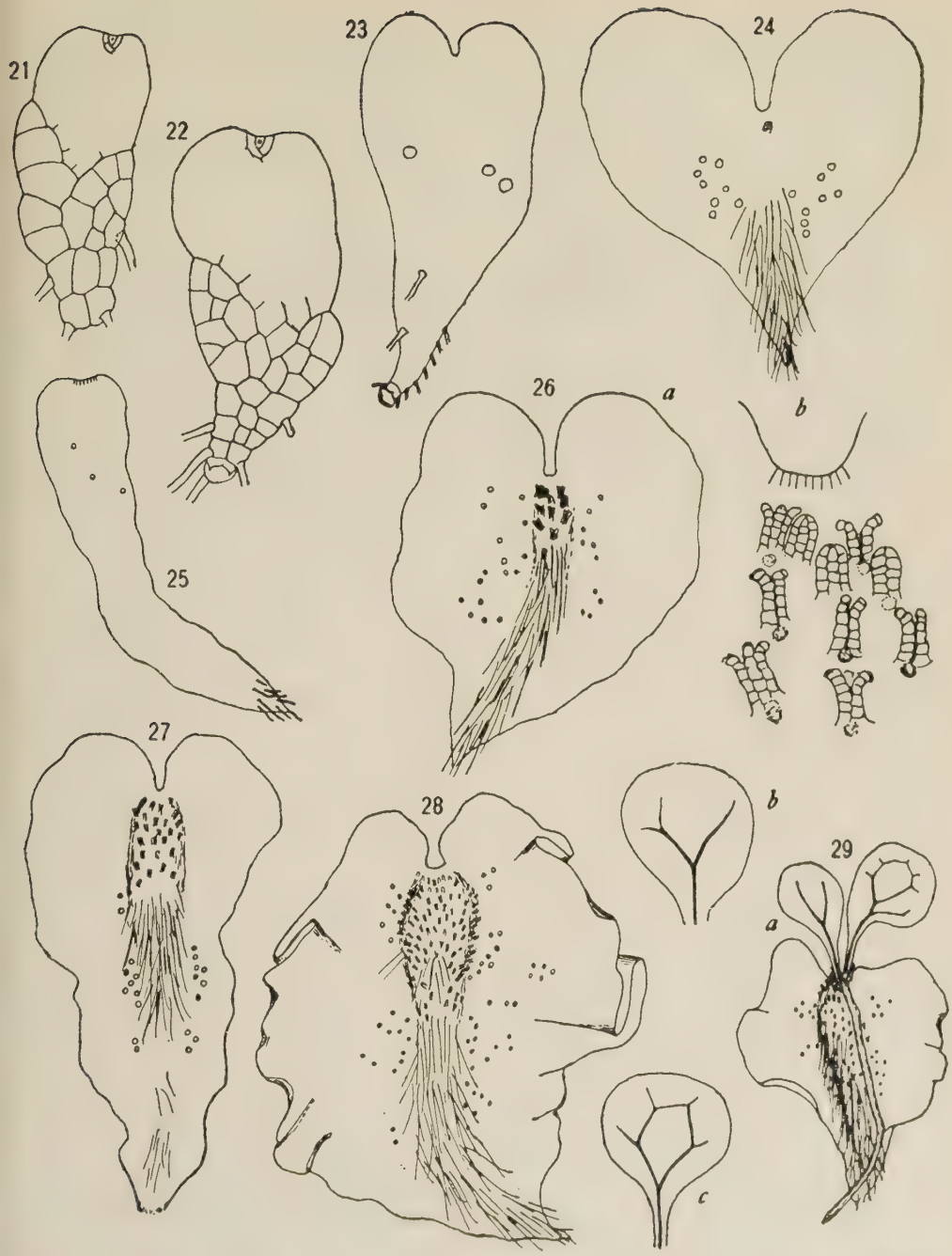
stage (Fig. 15). Occasionally the basal cell divides longitudinally or obliquely (Figs. 15, 21). Germination of the spore within the sporangium gives rise to longer cells than are found in prothalli which have germinated under more favourable conditions (Fig. 10), but even then the basal cell is short. The 9-celled filament in Fig. 8 grew in a clump over 3 months old. In our cultures there were few cases of branching filaments (Fig. 18) such as would be common in the higher ferns under similar conditions.

A wedge-shaped apical cell may appear early (Figs. 14, 19); in such cases its products often stand out conspicuously and suggest a lobing of the margin (Figs. 21, 22) which is usually lifted a little above the central axis. There is considerable variation in early stages; in some cases the walls in the plate are transverse and longitudinal (Figs. 16, 17) rather than oblique (Figs. 12, 14); the apical cell is delayed and may not appear until the gametophyte is more than three months old. This stage is probably passed over entirely in some gametophytes. The strong tendency of young prothalli in the plate stage to lift the lateral margins may sometimes suggest a thickened base. The folding in the early stage is rather like the early stage in *Matonia* (Stokey & Atkinson, 1952), but in later stages the wings are spread out (Fig. 62).

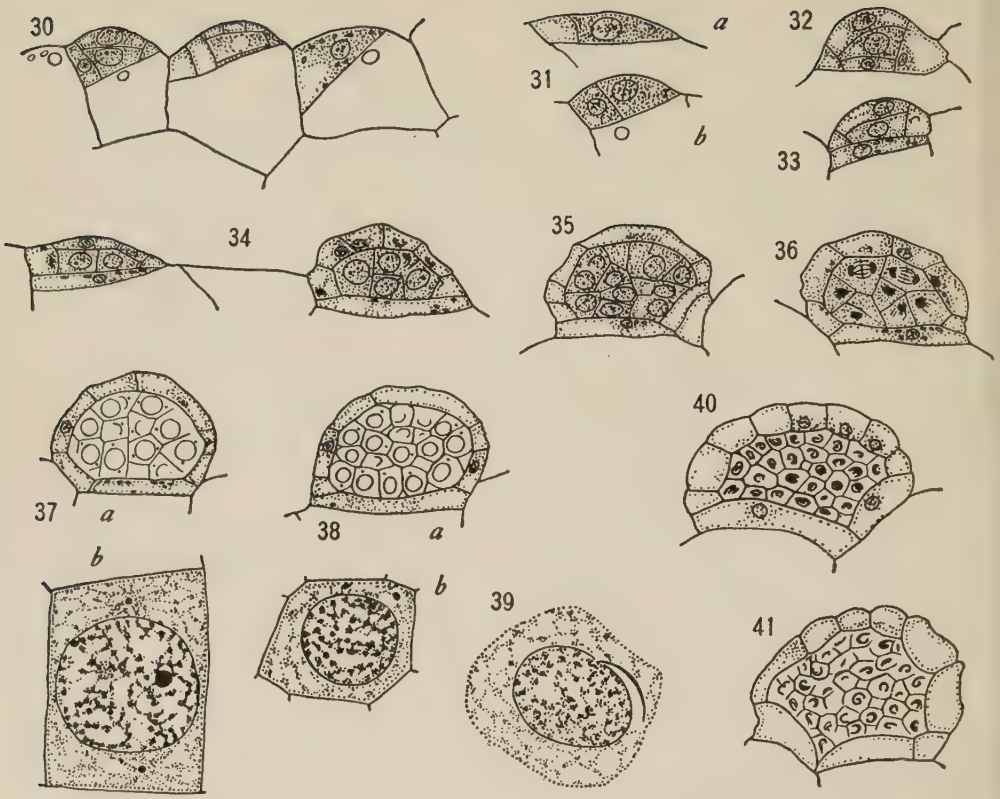
The thallus becomes more or less spatulate as it elongates, but the tip eventually becomes cordate (Figs. 23, 24, 26-28). The gametophyte is of slow growth, at least in cultures, and our material did not attain maturity until eight months after planting. The gametophytes during all this time appeared to be perfectly healthy and vigorous, dark green in colour — similar in shade to those of the Marattiaceae — at no time showing any yellowish tinge suggesting lack of sufficient light or any other deficiency. The wings are firm and flat with only slight irregularities along the margin and no ruffling except when old (Fig. 28). The midrib was not always thick in the early stages, and the thallus shown in Fig. 62 was only 6 or 7 cells thick at 15 months, but in others at 17 months the midrib was 11-15 cells thick (Fig. 61) and the heavy thickening was very close to the apex. The upper layer of the thallus is a much deeper green than the layers beneath. This is apparently true also of material growing in the open, as in Nakai's drawing, Fig. 7, it suggests an epidermis.

Nakai found an endophytic fungus in his prothalli, and suggested that the scarcity of gametophytes in nature may be related to the lack of the fungus. Our experience with gametophytes in culture suggests, however, that the fungus is not essential and that its presence is facultative and not obligate. It seems to us that the very slow development of the gametophyte is a more probable explanation of its scarcity. The growth of the elongating prothalli, although persistent, was so slow that at 18 months only a few had attained a length of 10-12 mm. The surface of the thallus is smooth with a heavy layer of cutin. The *Cheiropleuria* gametophytes were more resistant to attacks from fungi than were most of the other 40-45 species of ferns which we had in culture at the same time. It is possible that this resistance may be related to the heavy layer of cutin. No hairs appeared at any time on the gametophyte.

The rhizoids are dark reddish-brown or sometimes chocolate brown, exceptionally heavy and stout. Nakai said that not only did the presence of *Cheiropleuria* sporophytes identify the gametophytes, but the characteristic brown rhizoids were of assistance in collecting them where they were growing with a mixture of other ferns. The stout rhizoids appear in fair numbers on young gametophytes (Figs. 20-23), and while moderately abundant on old gametophytes they may be in discontinuous patches. They were not found near the archegonia and they do not form tangled mats such as are found on gametophytes which bear more abundant rhizoids or those of a softer texture. The germination of the spores in clumps leads to the crowding of the gametophytes in rosettes; this prevented the contact of the younger parts of the thallus with the substratum and may have influenced the distribution of rhizoids. In a few gametophytes some rhizoids showed septations.



FIGS. 21-29—Figs. 21, 22. Thallus in plate stage with apical cell, 4 months. ×90. Fig. 23. Thallus with 3 antheridia, 5½ months. Fig. 24. Thallus with antheridia and one archegonium, 8 months. × 12. Fig. 25. Thallus from crowded culture, 10 months. Fig. 26*a*. Thallus, 10 months; *b*, apical region of *a*; 26*a*, ×6; *b*, ×55. Fig. 27. Prothallus, 15 months. ×10. Fig. 28. Prothallus, 18 months. × 6. Fig. 29*a*. Gametophyte with young sporophyte; *b*, *c*, primary leaves from two other sporophytes. ×5.

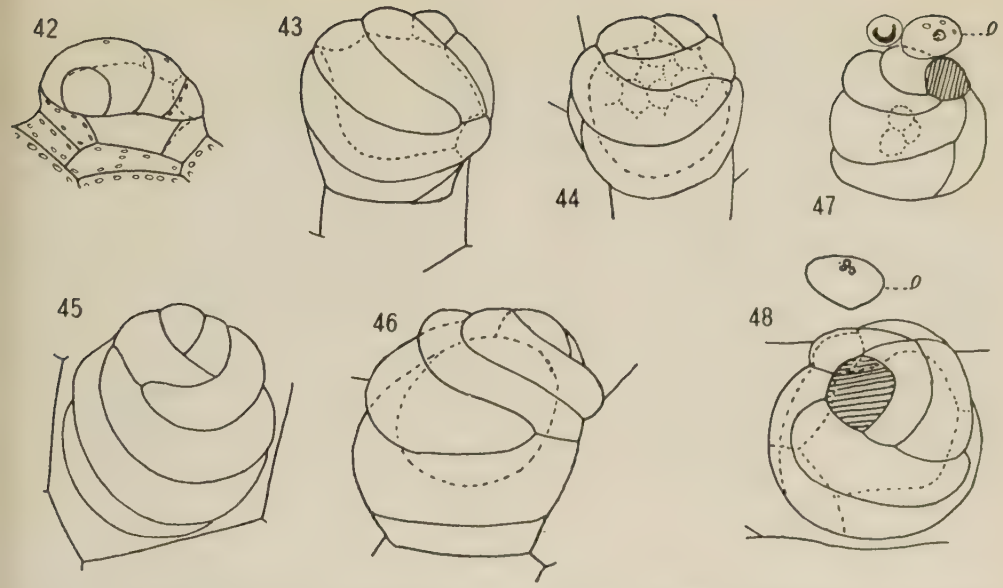


FIGS. 30-41 — Antheridia, median sections. Figs. 30-36. Early stages. Fig. 37*a*. Antheridium, spermatid mother cell stage; *b*, spermatid mother cell. Fig. 38*a*. Antheridium containing spermatids; *b*, spermatid. Fig. 39. Elongation of blepharoplast. Figs. 40, 41. Mature antheridium. ×320, except 37*b*, 38*b*, 39. × 2000.

Antheridium

Antheridia were first seen when the cultures were five months old (Fig. 23). They appeared on the ventral surface, and unlike most ferns in our cultures, they did not appear on the margin or dorsal surface. No ameristic male prothalli ever appeared in these cultures, but elongated ribbon-like thalli developing in crowded and unfavourable conditions may bear antheridia (Fig. 25). On mature gametophytes 10-18 months old both young and apparently functional mature antheridia were found on the wings at the same level as developing young archegonia (Fig. 26, 28).

The antheridium of *Cheiropleuria* is a very large and complicated structure. It develops from a wedge-shaped superficial cell on the wing or on the side of the cushion (Fig. 30); a series of divisions cuts off an outer layer of wall cells from the inner spermatogenous cells (Figs. 30, 31*a*, *b*, 32-37*a*, 38*a*, 40, 41). The latter divide to produce a large number of sperms (Figs. 40, 41), and the granules which are the forerunners of the blepharoplast (Fig. 39) appear in the spermatid mother cells (Fig. 37*b*) and the spermatid (Fig. 38*b*). The outer wall of the antheridium consists of a varying number of curved cells (Figs. 42-48) which show curvature early (Fig. 42). An opercular cell is formed which is thrown off at dehiscence (Figs. 47, 48). The antheridia are large and probably have the same degree of complexity as those of *Matonia*, *Dipteris* (Stokey, 1945), and the Gleicheniaceae



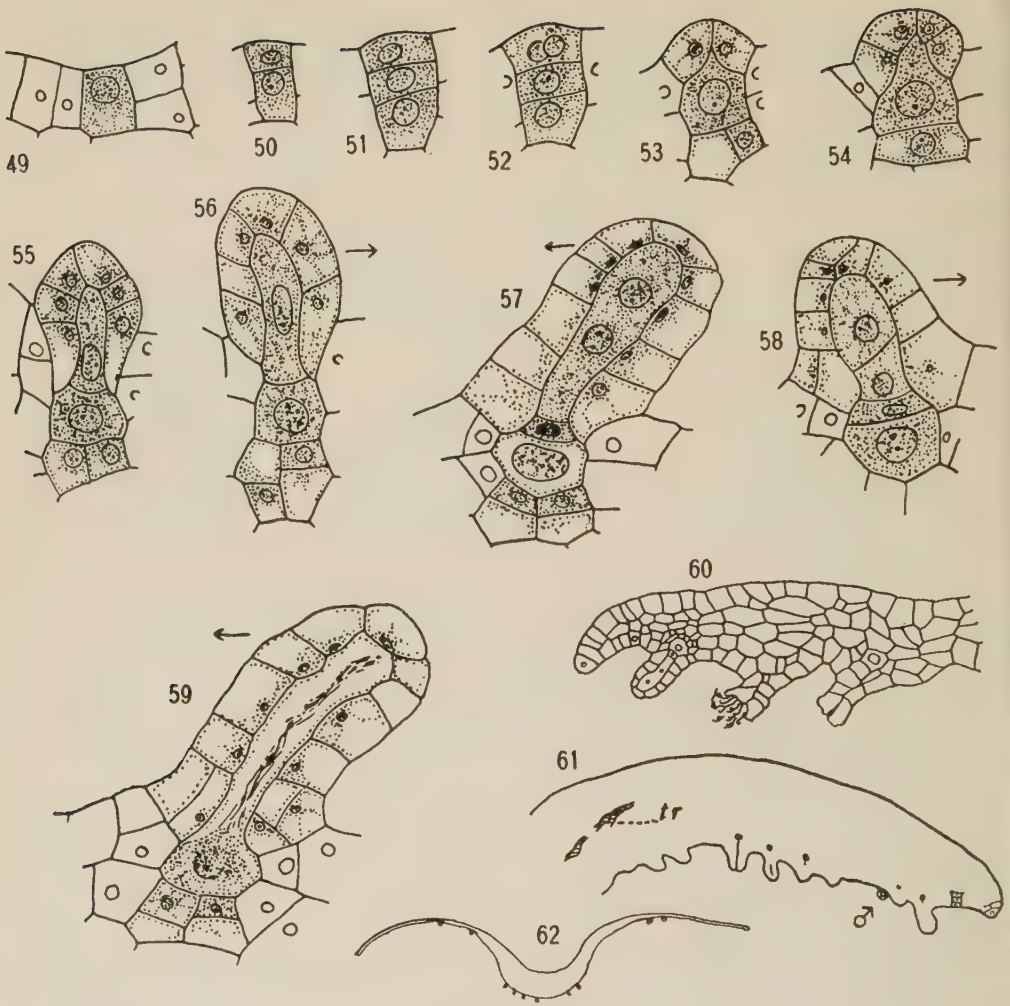
FIGS. 42-48 — Antheridium, external views. Fig. 42. Young antheridium. Figs. 43-46. Mature antheridium. Figs. 47, 48. Dehiscence; *o*, operculum. × 320.

(Stokey, 1950), but because of the arrangement of the outer walls they differ from these in external view. Seen from the side the cells of the outer wall in Gleicheniaceae, *Matonia*, and *Dipteris* appear twisted or of irregular shape. Those of *Cheiropleuria* appear to be of about equal width, and the lines of the anticlinal walls showing the relationship of these cells are roughly parallel to each other, and often stand nearly at right angles to the surface of the thallus (Figs. 42, 46). This places the opercular cell in a lateral position, usually on the side away from the cushion, with the result that dehiscence of the antheridium is difficult to see unless the thallus or antheridium is tilted.

Archegonium

The first archegonia were seen when the cultures were eight months old (Fig. 24), and from 10 or 11 months there was a continuous production of archegonia (Fig. 26a-28). Two features distinguish them: the neck is long, and it bends towards the apex of the thallus (Fig. 26b). It was never found inclined toward the base of the thallus although sometimes, especially as the thallus aged, the tilt was less pronounced or was directed toward the side of the midrib.

Development from an initial originating just behind the notch progresses in the usual manner (Figs. 49-56), and the mature archegonium possesses the usual complement of cells (Fig. 57). The length of the neck seems to depend more upon the length and size of the cells than upon the number (Figs. 55, 56); the neck is rarely more than five or six cells long — five on the side nearest the thallus and six on the more curved side (Fig. 57-59). The basal cells are somewhat irregular in size and position, and are sometimes pulled out of shape by the growth of the midrib. The ventral jacket of small cells with large nuclei surrounding the egg cell appears at maturity (Figs. 57, 59) and usually involves a division of the lowest neck cells. Sometimes the jacket consists of two layers of cells even when the neck is not open. In water on a slide the necks of some archegonia opened. In sectioned material sperms were found in the neck of the archegonia (Fig. 60), but the cultures were 15 months old before the first sporophyte was found. Several months later others appeared



FIGS. 49-62 — Archegonium. Figs. 49-56. Stages in development. Fig. 57. Mature archegonium. The arrow points towards the notch. Fig. 58. Archegonium with 2 neck canal cells. Fig. 59. Old archegonium. Figs. 49-59. × 320. Fig. 60. L.S. mature thallus. × 42. Fig. 61. L.S. apogamous thallus with tracheids, *tr*, 18 months. × 30. Fig. 62. T.S. thallus, 15 months. × 6.

(Fig. 29). In these sporophytes the simplest type of venation seen in a primary leaf is shown in Fig. 29*a*; the primary leaves *b* and *c* give variations from this, but in all cases there was a dichotomy of the vein at the base of the lamina. Both antheridia and archegonia continued to appear in cultures for more than two years. The necks of mature and old archegonia become massive and give a lumpy appearance to the ventral surface of the cushion (Fig. 60). Old thalli may reach a thickness of 11-15 cells, and the developing archegonia are crowded into a relatively small area near the notch. Here such anomalies as double eggs and two-celled neck canals (Fig. 58) may be found. On these ageing thalli the archegonia do not appear to be normal and functional.

Apogamy was found in one thallus 17 months old where in a longitudinal section a group of tracheids is present in the posterior region of the gametophyte (Fig. 61).

Discussion

Bower (1915, 1928) discussed the peculiar combination of characters in *Cheiropleuria bicuspis* with special emphasis on those which allied it to *Matonia* and *Dipteris* and, more remotely, to the Gleicheniaceae. He also considered its connection upwards as probably pointing toward *Platycerium*. Wagner (1952) took up the problem of *Cheiropleuria* in his work on venation in the fern leaf, and concluded that the combination of characters in the sporophyte marks it as a primitive fern and justifies its treatment as a family Cheiropleuriaceae. Nakai (1928) established the family on the basis of the sporophyte, and concluded from his later work on the gametophyte that its characters justified the family also. His figures and account of the gametophyte are not extensive, and do not agree with ours in all points. However, the characters brought out in the present investigation justify the family better than his account which was based on less material.

The family Cheiropleuriaceae was recognized by Ching (1940) and by Dickason (1946). Holttum (1949) placed *Cheiropleuria* in his family Polypodiaceae grouped with *Dipteris* as one of the primitive genera. Copeland (1947) placed it in his family Polypodiaceae as one of the primitive genera, following *Dipteris* and preceding *Christiopteris* and *Platycerium*; in his discussion of *Platycerium* he says that the affinity is not intimate.

We shall first consider the gametophyte of *Cheiropleuria* in relation to those of the three more primitive families, Gleicheniaceae, Matoniaceae and Dipteridaceae. It resembles those in some of its vegetative characters, differs in a few others, but is strikingly similar to them in reproductive structures and habits. It is like all three in the relatively slow germination of the spore, and also in having the tetrahedral spore found in *Matonia*, *Dipteris* and most of the Gleicheniaceae. It is like them in the slow development of the gametophyte, usually with a weak development of the filamentous stage and with early formation of a plate; it also has a mature thallus which is elongated rather than cordate or reniform although with a definite notch; and it agrees with them in having a relatively thick midrib. In vegetative characters it differs from the three families mentioned in the dark green colour of the thallus; it differs also in the flatness of the wings, which, although they may be more or less lifted, are not crisped and ruffled. It is like *Matonia* and *Dipteris* in the absence of hairs, but unlike the Gleicheniaceae in which there are a few hairs of a specialized type.

In *Cheiropleuria*, as in the three more primitive families, the gametophyte is slow in attaining maturity; antheridia did not appear on healthy well-grown thalli until they were 4-5 months old, and archegonia not until 8 months. There is also the continued production of antheridia on archegoniate gametophytes as is found in these families, but is unusual in the higher families. The antheridium is large with a many-celled complicated wall and a large sperm output; and the archegonia have relatively long necks which are inclined toward the apex of the thallus, never toward the base.

The vegetative characters of *Cheiropleuria*, such as the elongated thallus with thick midrib, may sometimes be found in the higher ferns, but only in very old gametophytes in which this stage is preceded by a well-defined cordate or reniform thallus. In general the gametophyte of *Cheiropleuria* shows less plasticity than those of higher ferns, in the scarcity of branching and in the lack of ameristic male gametophytes. But it is in the reproductive structures and habits that the most interesting characteristics appear. None of the unquestioned higher ferns, so far described, have the large antheridium with many-celled wall and large sperm output. There are, to be sure, in the higher ferns some species in which the gametophyte bears not only the common type of antheridium but at times a many-celled antheridium; these are of a many-tiered type (Stokey, 1951) and not the complex primitive type characteristic of such families as the Osmundaceae and Gleicheniaceae. Nakai (1933) stated that the antheridia show a great deal of similarity to those of the

Polypodiaceae, but his figures do not support that statement and indicate a much greater complexity in the wall.

The long archegonium neck is a character known only in primitive families. While in *Cheiropleuria* the neck does not develop as many cells as in the lower groups it attains an elongation suggestive of them. The curving of the neck toward the notch is known only in *Matonia, Dipteris* and the Gleicheniaceae. Nakai, however, described and figured the neck as straight; he considered the straightness of the neck one reason for removing *Cheiropleuria* from the Polypodiaceae.

In view of the usual association of *Cheiropleuria* with *Platycerium* we have made a study of the gametophytes of five species of *Platycerium* to see what evidence they give of relationship. The account of the gametophytes will be given in an accompanying paper, but a comparison will be made here. The gametophyte of *Platycerium* as exemplified by these five species, fits into the general pattern of the gametophyte of Copeland's Polypodiaceae, so far as they are known (Stokey & Atkinson, 1954). Germination of the spore is rapid (two to seven days), development of the thallus is rapid and there is more variation in form under varying conditions; the mature thallus is broadly cordate or reniform with a thin midrib; the sex organs appear early — antheridia in 20-30 days, archegonia in 40 days; antheridia and archegonia are not borne at the same time, antheridial production ceasing before that of archegonia begins; antheridia are small with the wall consisting ordinarily of three cells and occasionally a divided cap cell; the sperm output is low, 8-64 sperms; the archegonia have short necks curving toward the base of the thallus. *Platycerium* has multicellular branched hairs as well as simple hairs on the thallus.

The combination of primitive characters found in the gametophyte and the specialized characters which *Cheiropleuria* shares with *Matonia, Dipteris* and the Gleicheniaceae make it difficult to look upon *Cheiropleuria* as a member of the Polypodiaceae. In the structure and development of the gametophyte the gap between *Cheiropleuria* and the advanced ferns with which it has been associated is very much greater than that between it and the more primitive ferns.

Summary

The gametophyte of *Cheiropleuria bicuspis* develops very slowly; in a healthy vigorous culture it may require five months to develop antheridia and eight months for archegonia. On germination there is a short filament, rarely a plate, which grows into a narrowly cordate dark green thallus with a midrib which may become 11-15 cells thick. No hairs are formed. Antheridia are large and have a many-celled complicated wall; a cap cell is discharged at dehiscence. Archegonia have long necks inclined toward the apex of the thallus. One gametophyte showed apogamy — tracheids in the thick midrib. The gametophyte justifies Nakai's family Cheiropleuriaceae, as it has much more in common with those of the Gleicheniaceae, Matoniaceae and Dipteridaceae than with those of the higher ferns.

Part of this investigation was carried out by the senior author at the Marine Biological Laboratory, Woods Hole, Mass.

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STUDIES IN THE FAMILY ANACARDIACEAE — I. VASCULAR ANATOMY OF THE FLOWER OF *MANGIFERA INDICA* L.

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Introduction

Mangifera indica, the common mango, is a well known and widely cultivated plant in India. Its original home is considered to be India and Malaya or Indo-malayasia (Mukherjee, 1949; Lawrence, 1951). Now it is grown in almost all tropical sectors and also the temperate and sub-temperate zones of the world for its delicious edible fruit. In India, its economic importance is so great that it is familiar to all classes of people in various kinds of food articles.

The plant belongs to the tribe Anacardiae of the Anacardiaceae in the order Sapinadales. Hooker (1879) assigned about 30 species to this genus, but very recently Mukherjee (1949) has estimated 41 valid species which are put into two sections. *Mangifera indica*, with over a thousand varieties, is placed in group I of the first section.

Juliano and Cuevas (1932) described the morphology of the mango flower and Venning (1948) investigated the ontogeny of its laticiferous ducts. Besides this, some other aspects have also been touched by Maheshwari (1934) and Mustard and Lynch (1946). It appears that the family has suffered considerable reduction particularly in the number of stamens and perhaps in the number of carpels (cf. Rendle, 1926). Since vascular anatomy of the flower has often yielded useful information in cases of reduction (see Puri, 1951), it was considered worth while to study the vascular anatomy of the flower of *Mangifera indica* which is available here.

Materials and Methods

Buds and flowers of all stages were fixed in F.A.A. and dehydrated and embedded in the usual manner. Serial microtome sections, 10-14 microns thick, were cut and stained in crystal violet and erythrosin. Buds and flowers were also studied under a dissecting microscope.

Observations

External Morphology — Depending upon the variety, the size of the plant varies from a medium sized to a fairly tall tree. In this part of the country it blooms from February to April. The flowers, which are produced in great profusion, are small, bracteate, sub-sessile, greenish-yellow, pentamerous polygamous, sweetly fragrant and arranged in terminal somewhat tomentose panicles 6-12 inches long. Hermaphrodite and male flowers occur together in the same panicle.

There are 4-8 petals, 5 being the commonest number. Every petal has 3-5 orange- or pink-coloured ridges on its upper (ventral) surface (Figs. 9, 12). In between the petals and stamens, there is a thick, fleshy and annular disc which has approximately as many lobes as the number of petals. Of the five stamens which appear to be emerging from the disc only one, rarely two, are fertile while the others are staminodes often represented only by filaments of different heights. The gynaeceum is usually monocarpellary with a single style and simple stigma. The single anatropous ovule is borne on a long ascending funicle attached sub-basally.

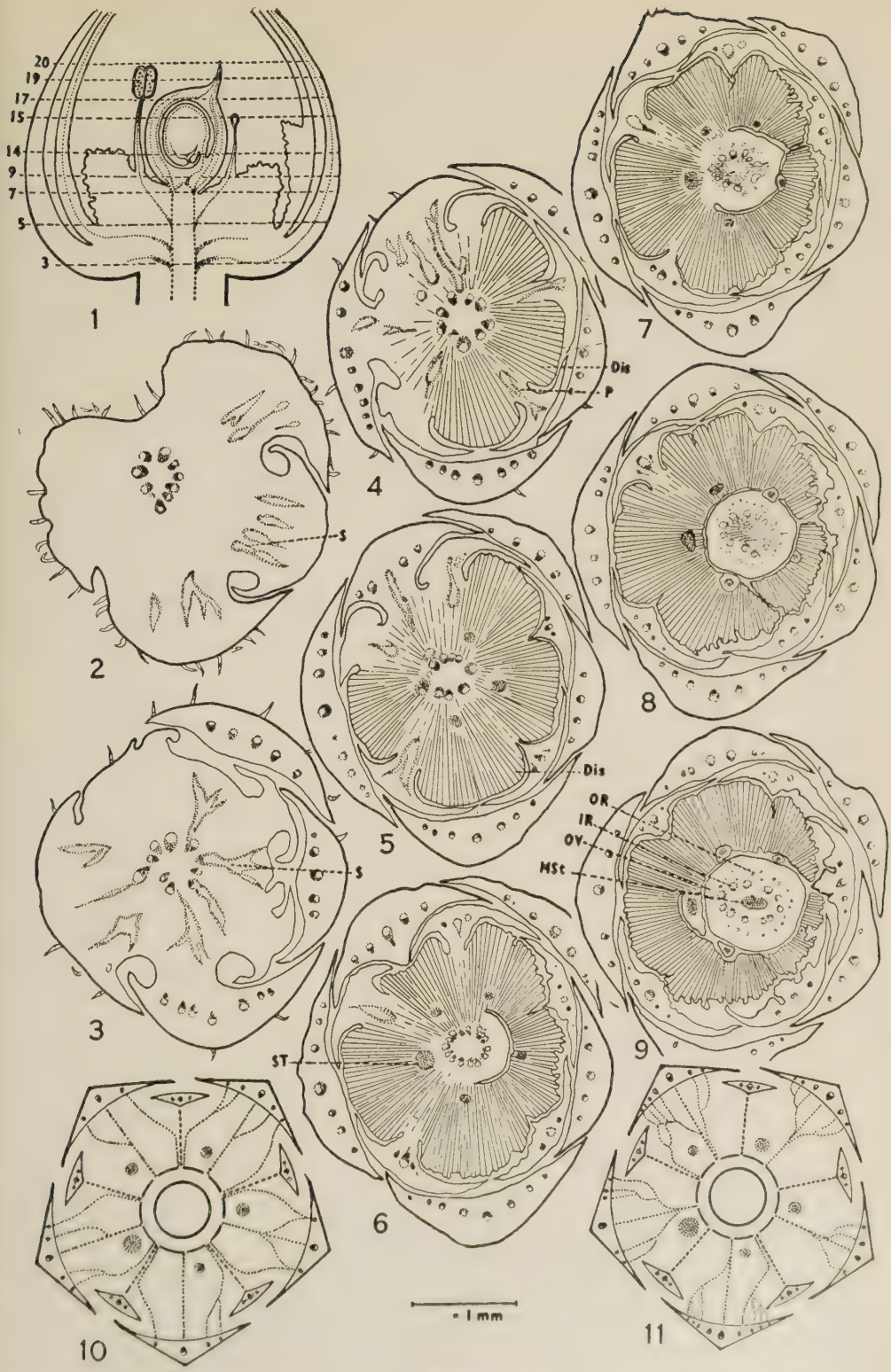
Vascular Anatomy — A transverse section of the peduncle in the lower region shows a ring of about 10 somewhat unequal vascular bundles. There is a conspicuous laticiferous canal within the phloem of every vascular bundle. One of the bundles gives off a trace to the emerging bract. This is followed by some traces from either side which organize the vascular supply of the axillary bud, subtended by the bract. At the base of the pedicel of a single flower, only 5 bundles are visible which divide and form a ring of about 10 bundles of unequal size. At this level the hairy coating on the pedicel becomes very dense.

The pedicel stele gives off one trace to each sepal in order of its development (Fig. 2). Each trace, while still within the receptacular cortex, gives off 1, 2 or more branches on either side and thus forms a band of 3, 5 or more bundles in transverse section (Figs. 3-5, 10). In other cases the midrib and the lateral bundles of a sepal are derived from three different sources. The median trace is obtained directly from the parent stele, whereas the lateral traces are derived from petal strands on either side (Figs. 10, 11). These two conditions regarding sepal supply may be found in different flowers or sometimes in the same flower. There is, therefore, no hard and fast rule regarding the sepal laterals. The outer sepal in many flowers is slightly bigger in size and receives a larger number of traces. Each bundle in the sepal has a laticiferous canal in the phloem, which is continuous from the bundle of the receptacle.

Almost simultaneously with the departure of the sepal medians, there diverge out the five petal strands which soon break up tangentially into two each (Figs. 4, 10, 11). The inner of these daughter bundles form the midrib bundles of petals while the outer may split again into two lateral veins each of which enters the adjacent margins of sepals or may enter one of the sepals without splitting (Fig. 10). Within the petal each midrib bundle usually gives off one lateral branch on either side (Figs. 5, 6). Thus every petal comes to have three bundles which

→

Figs. 1-11 — Fig. 1. Semi-diagrammatic l.s. of a hermaphrodite flower showing vascular supply (dotted lines) to different organs. The numbers on the left indicate levels at which transverse sections have been shown in figures bearing the same number. Fig. 2. T.S. flower showing peripheral parts of sepal traces *S*, the sepal bases being saccate. Fig. 3. Connection of sepal traces *S* with the central stele. Fig. 4. Petal traces *P* are going out; note appearance of disc *Dis*. Figs. 5, 6. Stamen traces *ST* have diverged out and the base of the gynaeceum has started separating from the disc. Note difference in size of staminal traces. Figs. 7, 8. The base of the gynaeceum has separated from the disc and the bundles of the central stele are giving off traces both inside and outside. Fig. 9. The inner branches have fused into a prominent medullary strand *MSt* in centre of ovary *OV*, while the outer bundles divide and organize into two more or less distinct rings, outer *OR* and inner *IR*. Fig. 10. Diagrammatic representation to show variations in the vascular supply of sepals and petals in a male flower. Fig. 11. Same, for a hermaphrodite flower.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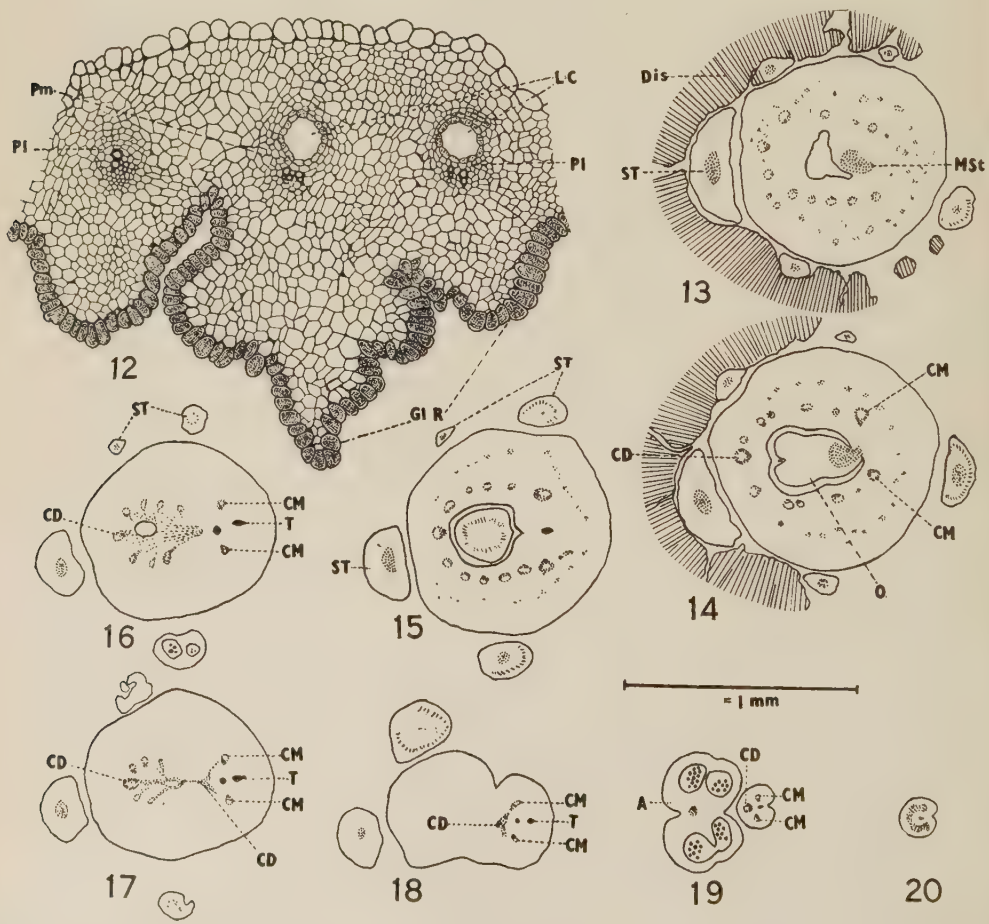
Figs. 1-11.

may later on undergo further divisions (Figs. 7-9). Like sepals each petal trace is accompanied by a laticiferous canal which is clearly seen in the petal midrib bundle and may also be present in laterals (Fig. 12). Venning (1948), however, denies the presence of laticiferous canals in the petal laterals. The glandular ridges on the inner sides of petals do not have any vascular tissue (Figs. 9, 12).

Soon after the separation of the petals, the glandular disc also breaks up into five more or less distinct lobes alternating with the petals (Figs. 5-8). The lobes are much more distinct in male flowers than in the hermaphrodite flowers. The ridges on the petals are glandular and they appear to be mere continuations of the disc which seems to spread out on petal surfaces at lower levels (Figs. 1, 5-7).

Just when the petals have started separating but the disc is still in tact, five bundles are seen outside the central stele (Figs. 5, 6). These are the staminal



FIGS. 12-20 — Fig. 12. T.S. of midrib region of petal showing the three glandular ridges *Gl R* and one median *Pm* and two lateral *Pl* bundles. (×126). The lateral bundle on the right also shows a laticiferous canal *LC*. Fig. 13. Medullary strand being pushed to one side as it were by the locule. Fig. 14. In the inner ring three bundles, the carpellary dorsal *CD* and the two carpellary marginals *CM* have become more distinct than the rest. The medullary strand is "passing" into the ovule *O*. Figs. 15, 16. Transmitting tissue *T* has appeared in between the two marginal bundles. The carpellary dorsal *CD* is preparing to cross over to the right. Figs. 17, 18. Carpellary dorsal has crossed over to the right. Fig. 19. Fertile anther *A* and style. Fig. 20. The three carpellary bundles fuse in the stigmatic region.

traces which, unlike sepal and petal traces, are devoid of laticiferous canals. They vary considerably in size. The largest supplies the single fertile stamen while the others, which are smaller, supply the staminodes having varying heights (Figs. 1, 6). They usually alternate with the petals and appear to be embedded in the tissue of the disc (Figs. 6-9). Further up, the disc disappears and the staminodes emerge out (Figs. 14-18). Occasionally they may show imperfectly developed pollen grains.

In some cases ten staminal traces are clearly seen in the receptacle but only five enter the stamen and staminodes, while the others disappear at varying heights appearing merely as vascular stubs of other staminodes which no longer exist.

After the separation of the stamen traces, there are left 10-15 bundles in the central stele (Fig. 6). These undergo repeated divisions sending out branches on the inner as well as the outer side and form, for a time, a more or less confused mass of vascular tissue (Figs. 7, 8). But very soon this plexus differentiates into three more or less distinct regions: (i) the outer ring *OR*, (ii) the inner ring *IR*, and (iii) the medullary strand *MSt* (Fig. 9).

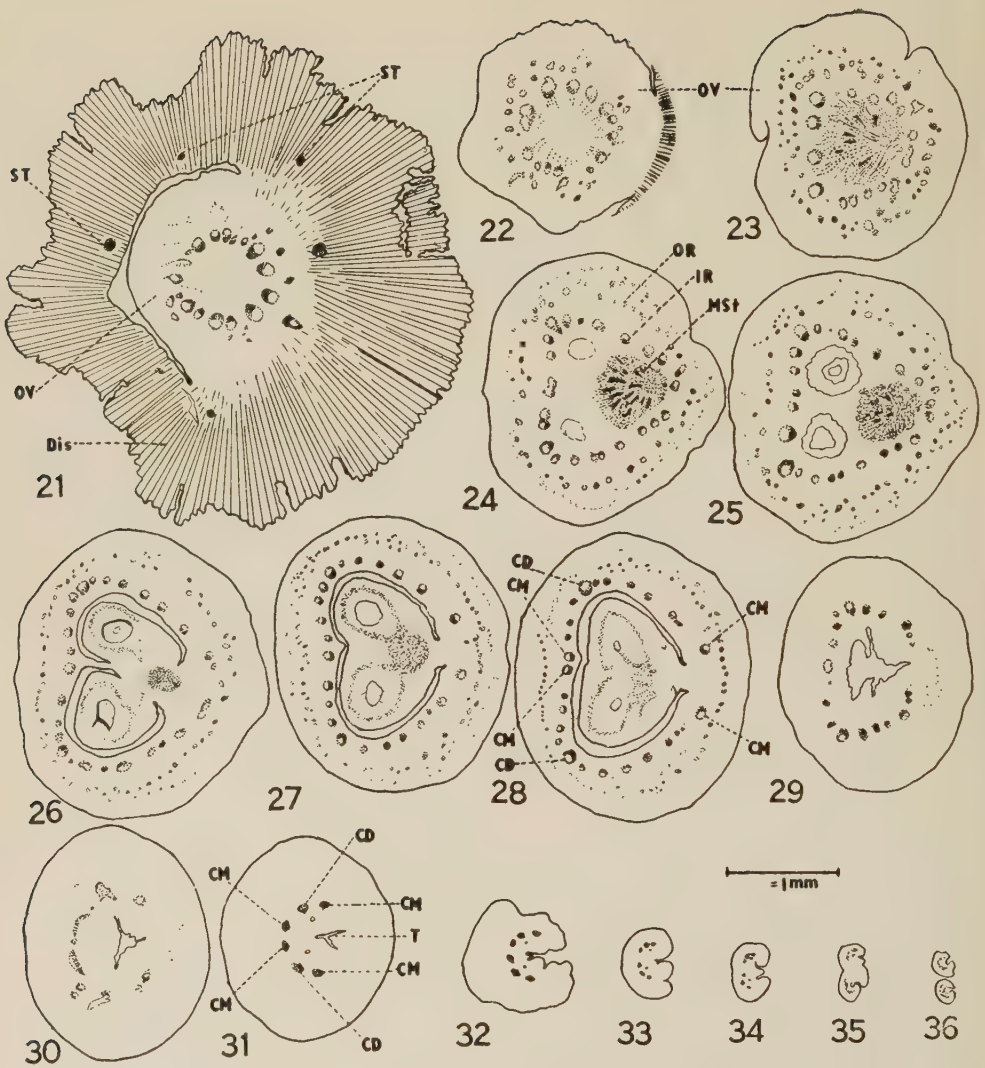
The outer ring consists of twenty or more small unequal vascular bundles arranged more or less irregularly. They continue up in the outer region of the ovary wall and disappear towards the end of the ovary. The number and size of these bundles appear to vary in different varieties and they seem to determine to some extent the quality of the fruit. The so-called " Bombay " variety, for instance, has larger and more numerous bundles and is considered to be inferior to the so-called " Langra " in which the peeling is thin and flesh is without too many of these vascular threads.

The inner ring, which is actually the parent ring for all the three regions, consists of 10-12 more or less prominent vascular bundles. Depending upon the outline of the young fruit it may be oval or circular in cross-section. In this ring three bundles become more prominent than the rest (Figs. 14, 15). One of these is situated on the anterior side and is probably the dorsal bundle of the carpel. The other two are situated on the other side and are nearer to one another than either of them is to the dorsal (Figs. 14, 16). These are described here as the two marginal bundles of a single carpel. Whether they are really ventral bundles or secondary marginals will be discussed later. Venning (1948), in his Fig. 6, has labelled them as " Lateral bundles " in which he did not observe the laticiferous canals, but I have seen distinct laticiferous canals, running up to style or even up to stigma in some cases. As the top of the ovary is approached the dorsal bundle migrates over to the other side and comes to lie on the ventral side in between the two marginal bundles (Figs. 1, 17). These three bundles alone continue into the style while all the rest disappear in the ovary wall (Figs. 1, 18, 19). Enclosed within them there soon appears a patch of transmitting tissue which ends at the tip of the stigma (Figs. 18-20).

The original main stele gives off several branches which diverge more or less horizontally inward and form a central plexus (Figs. 1, 8, 9). This differentiates into what has been called here as the central medullary strand. Further up this migrates slightly to one side and enters the funicle (Fig. 1, 13). During its upward course the ovular trace gives off many small branches. On reaching the chalaza it crosses over to the other side and descends down the integument right up to the micropyle region (Fig. 1).

The vascular plan of the male flowers is similar to that of the hermaphrodite flowers in so far as the supply of the sepals, petals and stamens is concerned. As the pistillode is poorly developed its vascular supply also is correspondingly reduced. The central stele after the departure of stamen traces consists of ten or fewer bundles which disappear after traversing a short distance.

Beside the normal monocarpellary condition, some cases were observed of bicarpellary gynaecia which may be syncarpous or apocarpous. In the bicarpellary syncarpous flower the vascular supply to the sepals, petals and stamens is normal. At the level of the ovary the bundles in the central ring increase in number and



Figs. 21-36 — Serial transverse sections of a bicarpellary syncarpous gynaeceum. In the lower region the ovary is bilocular but in the upper region the septum disappears and it becomes unilocular. Note the double vascular supply in Figs. 28-36. (Abbreviations same as in previous figures.)

soon differentiate into the usual three regions (Figs. 21-24). In the sections figured, the outer ring shows a differentiation into two or more zones of irregular and unequal vascular bundles which supply the mesocarp (pulp) and the epicarp of the fruit as in normal cases (Figs. 24-26). The inner ring with about 25 bundles becomes slightly triangular in transverse sections (Figs. 24-28). From this there differentiate two dorsal and four marginal bundles corresponding to the two carpels (Figs. 28-31). These six bundles arranged in two more or less distinct groups enter the style while all the rest disappear towards the apex of the ovary (Fig. 31). Each of these groups consists of a dorsal and two marginal bundles. This is clearly indicated by the fact that they enter into separate stigmatic

lobes which represent separate carpels (Figs. 31-36). The medullary strand, which is produced in the same manner as in monocarpellary gynaecia, splits into two strands, one entering each of the two ovules (Fig. 28). Before this the two ovules are situated in separate locules, but soon the septum between them breaks off and disappears and the two cavities merge into one (Figs. 25-28).

In some cases after the departure of the sepal, petal and stamen traces the central stele which consists of 20-25 bundles becomes pinched off into two more or less equal steles. These constitute the vascular supply of two separate carpels which are perfectly normal in their structure.

Discussion

Reduction in Floral Parts — From the foregoing account it will be clear that the mango flower is still in the process of change and that the structural stability which is met with in some other plants has not so far been attained. Besides, the flower appears to be undergoing considerable reduction. For instance, the three sepal bundles may be derived from a single or three different gaps. Similarly the vascular supply of the petals is also variable.

Although the flowers are essentially hermaphrodite they are tending to become unisexual. In most twigs male flowers seem to predominate over the hermaphrodite ones. Besides, the number of stamens and carpels also appear to be undergoing reduction. Generally there are five staminal traces but in one case an additional whorl of five vascular stubs was observed. This can be interpreted to mean that the ancestral mango flower had at least two whorls of stamens. In the present-day form not only has one of the two whorls been completely suppressed but in the surviving whorl 3 or 4 stamens have been reduced to sterile staminodes. In the same way the monocarpellary condition appears to have been derived from a tricarpellary condition.

Interpretation of the Carpellary Bundles — Many vascular bundles traverse the ovary region but there are only three whose interpretation is important from the point of view of the form of the carpel. These are the two "marginal" bundles and the central medullary strand. There are two possible ways in which these can be interpreted: (i) either the marginal bundles are true ventral bundles of the carpel and the central medullary strand is a branch therefrom for supplying the ovule, or (ii) they are secondary marginals and the central medullary strand represents the fused ventral bundles which disappear in furnishing one or more ovular traces. It is difficult to decide the point but I am inclined to consider the second alternative to be more satisfactory. This is supported by the following considerations: (1) The central medullary strand is derived from bundles all around and not from any specific bundles. It cannot, therefore, be regarded as a branch from the lateral bundles. (2) The lateral bundles are not concerned in the ovular supply and hence they cannot reasonably be regarded as ventral bundles (see Puri, 1952).

Summary

1. The paper deals with the vascular anatomy of the flower of *Mangifera indica* L.

2. The sepals and petals are normal with regard to their supply but the origin of the lateral traces of sepals has been observed to vary considerably. Sometimes they may arise from petal strands and sometimes from sepal dorsals, or from both.

3. There are 5 staminal traces of variable size. Of these the largest supplies the single fertile stamen and the rest enter the non-functional staminodes. Additional vascular traces have sometimes been observed to continue for a short distance; these represent missing staminodes.

4. The five-lobed glandular disc shows no vascular elements and appears to be a receptacular outgrowth.

5. In hermaphrodite flowers the carpel receives, beside the one dorsal and two laterals, many other bundles which continue up to the end of ovary. The single ovule is lateral and receives one prominent bundle which is formed by traces coming in from different sides.

6. Attention has been drawn to the probable reduction in the number and fertility of the stamens and in the number of carpels.

7. The nature of the carpellary bundles is discussed and it has been tentatively concluded that the laterals in the carpellary wall are secondary marginals and that the real ventrals are short-lived and continue as ovular traces.

I am greatly indebted to Dr. V. Puri for initiating me into research and for his constant help and guidance during the progress of this work. I am also thankful to Mr. Y. S. Murty and other colleagues for encouragement and sustained interest.

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THE STRUCTURE AND DEVELOPMENT OF EXTRA-FLORAL NECTARIES IN *DURANTA PLUMIERI* JACQ.

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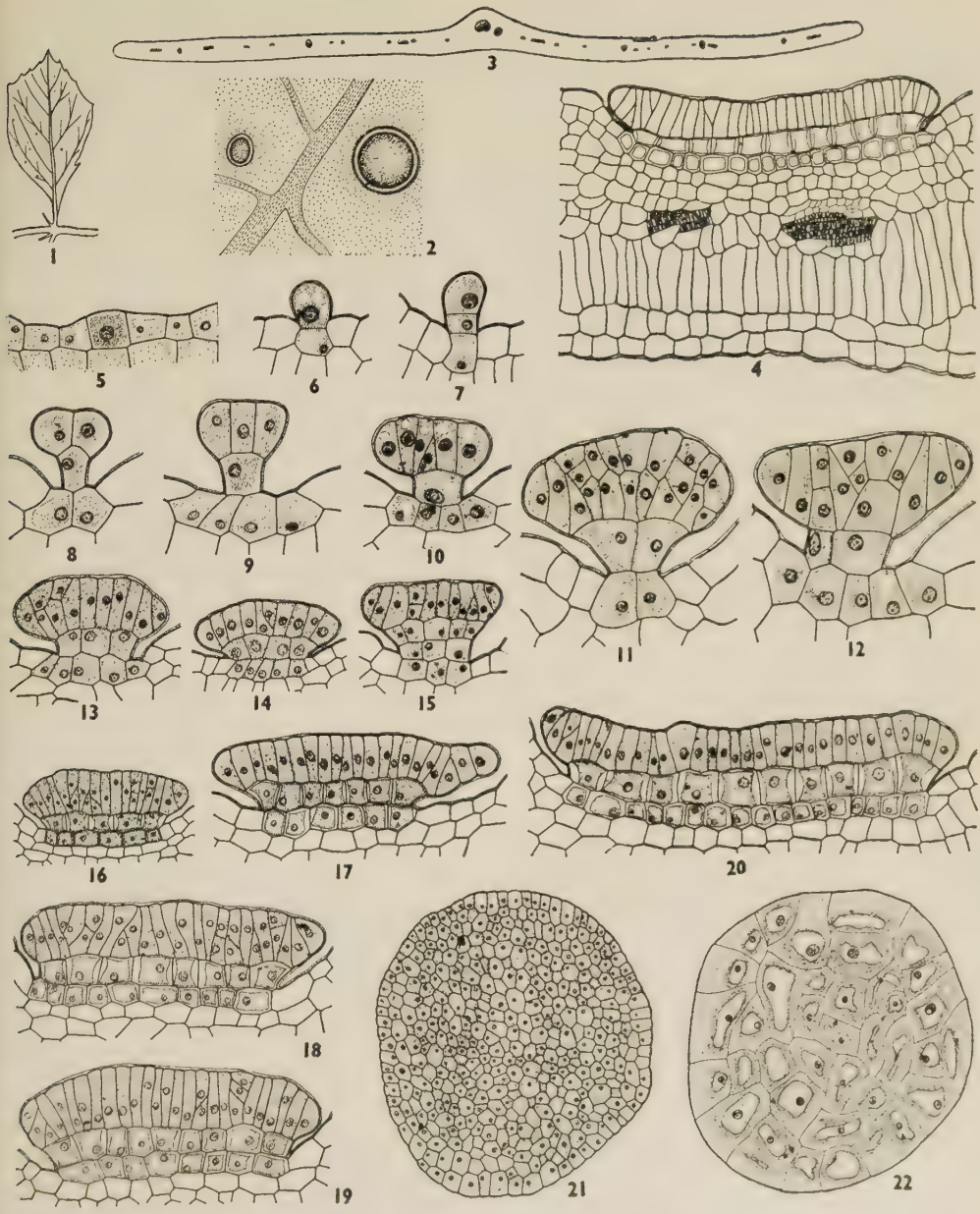
Extra-floral nectaries are known to be of widespread occurrence among flowering plants on the stem, leaf surface, leaf margin, petiole, stipule and base of flower. In the family Verbenaceae they have been recorded on the surface of the leaf or petiole of species of *Amasonia, Baillonia, Callicarpa, Casselia, Citharexylum, Dipyrena, Duranta, Lampaya, Monochilus, Raphithamnus, Stachytarpheta* and *Clerodendron* (see Metcalfe & Chalk, 1950). However, our knowledge of the ontogeny of these organs is rather scanty. The present work deals with their development in a common hedge plant, *Duranta plumieri*, in which the glands are scattered all over the lower (Fig. 1) and occasionally even the upper surface of the leaf. To the naked eye, they are visible only when nearly mature. In surface view the nectaries appear under the low power of the microscope as circular or ovoid bodies borne in a slight depression (Figs. 2, 3).



FIGS. 1-22 — Fig. 1. Lower surface of leaf showing location of extra-floral nectaries in the form of large dots. × 1. Fig. 2. Part of leaf magnified to show nectaries in surface view. × 90. Fig. 3. V.S. leaf showing nectary on right. In this as well as other drawings the lower surface of the leaf is shown upwards. × 47·5. Fig. 4. Same, part of leaf magnified. × 650. Fig. 5. Epidermal nectary initial about to protrude out as a vesicle. × 1500. Fig. 6. Same, after transverse division. × 1500. Fig. 7. Basal, stalk and body cells. × 1500. Figs. 8-19. Stages in development of the nectary. Figs. 8-12. × 1500. Figs. 13-15. × 900. Fig. 16. × 650. Figs. 17-19. × 900. Fig. 20. Nearly mature nectary. × 900. Fig. 21. T.S. of nectary passing through body region. × 900. Fig. 22. T.S. through stalk region showing the conspicuously thickened cell walls. × 900.

DEVELOPMENT—The nectaries originate from epidermal cells usually situated on the lower surface of the leaf. A cell, which is destined to develop into a nectary, is characterized by its larger size, more prominent nucleus and denser cytoplasm (Fig. 5). It becomes papillate and divides periclinally to form two daughter cells (Fig. 6), of which the upper may be designated as nectary mother cell and the lower as basal cell. The former divides again, resulting in what may be called the primary stalk cell and the body cell (Fig. 7). The body cell gives rise to the main body of the nectary; the stalk cell forms a short but broad stalk; and the derivatives of the basal cell form a layer very similar to that derived from the stalk cell. Figs. 8-10 show stages in anticlinal divisions of the body cell and the basal cell, while the middle or stalk cell has so far remained undivided. Later divisions in the body cell are irregular and occur in different planes, resulting in a broad saucer-shaped head. The primary stalk cell also divides anticlinally (Figs. 11-20) producing a plate of cells nearly as broad as the main body of the nectary. Sometimes, it first divides periclinally and then anticlinally so as to result in two layers of cells. Simultaneously, the basal cells (Figs. 8-20) also undergo vertical divisions so as to keep pace with the breadth of the stalk region. They remain in continuation with the leaf epidermis but differ from the epidermal cells in their denser cytoplasm and larger nuclei. They simulate the stalk cells not only in their staining reaction, but also in the subsequent development of thickenings.

STRUCTURE AND FUNCTION — The nectaries are patelliform (Figs. 3, 4) and lie in a shallow depression. Similar disk-shaped, slightly depressed glands are found in *Adenocalymma* (see Metcalfe & Chalk, 1950), a member of the Bignoniaceae. The body of the nectary is represented by a palisade-like layer of 30-40 cells (Figs. 4, 20), whose outer walls become thickened. There is a thin cuticular covering running in continuation with the general surface of the leaf. There is a two- (Figs. 4, 20) or sometimes three-layered (Fig. 15) stalk. The stalk cells are rectangular and their anticlinal walls are thicker than the periclinal walls. The cells of the gland proper are long, narrow and palisade-like and stain densely (Fig. 20). According to the classification, suggested by Zimmermann (1932) on the basis of morphological and anatomical features, they are extra-floral nectaries of the depressed type. However, since they possess a two-layered stalk and the body of the gland consists of long, palisade-like cells, they can also be referred to as extra-floral nectaries of the surface type. A transverse section of the nectary in the body region consists of small, parenchymatous cells with minute nuclei (Fig. 21). The cells in the stalk region (Fig. 22) are larger and have conspicuous thickenings. In *Clerodendron* (see Metcalfe & Chalk, 1950), each gland consists of a palisade-like epidermis which is subtended on the inside by a layer of polygonal cells whose vertical walls are suberized.

The primary function of these extra-floral organs is conceived to be the excretion of superfluous fluids. They stand in close relation to the vascular system. When the atmosphere is sufficiently humid, a minute globule of sugary sap is seen exuding out of the nectary. Sugar secretion by extra-floral nectaries has been demonstrated by Zimmermann (1932) in 40 families (including two grasses); it is held to be probable in 10 families and doubtful in 11 families.

Summary

The present work deals with the development and structure of extra-floral nectaries in *Duranta plumieri*.

They are found in a shallow depression on the lower surface of the leaf and are epidermal in origin.

At maturity, the nectary consists of a body made up of long, narrow, palisade-like cells subtended by a two-layered or rarely three-layered stalk. The stalk cells develop conspicuous thickenings on the radial walls.

The glands are patelliform or ovoid in shape. On a humid day a very minute globule of sugary sap comes out of them.

It gives me great pleasure to express my gratitude to Prof. P. Maheshwari for his guidance and criticism. Thanks are due to the Council of Scientific & Industrial Research, New Delhi, for a grant in connection with Prof. P. Maheshwari's scheme on " Flora of Delhi State ".

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MORPHOLOGICAL DEVELOPMENT OF THE ROOT AND STEM NODULES OF *AESCHYNOMENE INDICA* L.

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Introduction

Bacterial nodules are known to be of general occurrence on the roots of leguminous plants. Swellings, anatomically different from typical nodules, have been reported on the roots of a zygophyllaceous weed, *Tribulus cistoides* (Allen & Allen, 1950b), but they do not contain any bacteria[1]. Only rarely do bacterial nodules occur on stems and leaves.

Although the developmental morphology of the root nodules of several herbaceous legumes has been studied, nodules on woody species of the family have received less attention. Jimbo (1927) described the physiological anatomy of the persistent root nodules of *Wistaria sinensis*; and Harris, Allen and Allen (1949) have investigated the morphology of the bacterial nodules of *Sesbania grandiflora*. The present work on *Aeschynomene indica* was undertaken not only because it is a woody plant but also because of its being exceptional in having nodules on the stems as well as the roots.

Allen and Allen (1950a) have recently published a review on bacterial nodules from which it appears that there are wide differences in the mode of infection, longevity and position of these structures. The root nodules of *Wistaria sinensis* (Jimbo, 1927) show ring-like formations, each of which is said to represent an annual growth. In *Sesbania grandiflora* (Harris, Allen & Allen, 1949) also 8-12 months old plants showed large lobed nodules with emerging rootlets. The nodules are usually cortical in origin and the rhizobia enter through the root hairs (see Thornton, 1930; Bond, 1948). *Arachis hypogea*, the peanut, is a notable exception in having endogenous (pericyclic) nodules, the infection occurring through the ruptured tissue at the site of emergence of the lateral roots (Allen & Allen, 1940).

1. *Tribulus terrestris*, which occurs locally, was found to have no nodules of any kind.

Material and Methods

The material for this study was collected from the banks of the Najafgarh canal, Delhi. Small pieces of the roots and stems were fixed in formalin-acetic-alcohol and Nawaschin's fluid. The usual methods of dehydrating and imbedding were followed. Sections were cut 8-12 μ thick and stained in safranin-fast green, Heidenhain's iron-haematoxylin and Flemming's triple stain. Smears were stained by Gram's I-KI method.

Observations

The nodules commonly occur singly and are widely distributed on the roots. Root as well as stem nodules are alike in shape and size, in being round and flat, with an uneven surface and a broad basal attachment (Fig. 9). In the roots the nodules as well as the periderm are of pericyclic origin; in the stem they arise from the outer cortex.

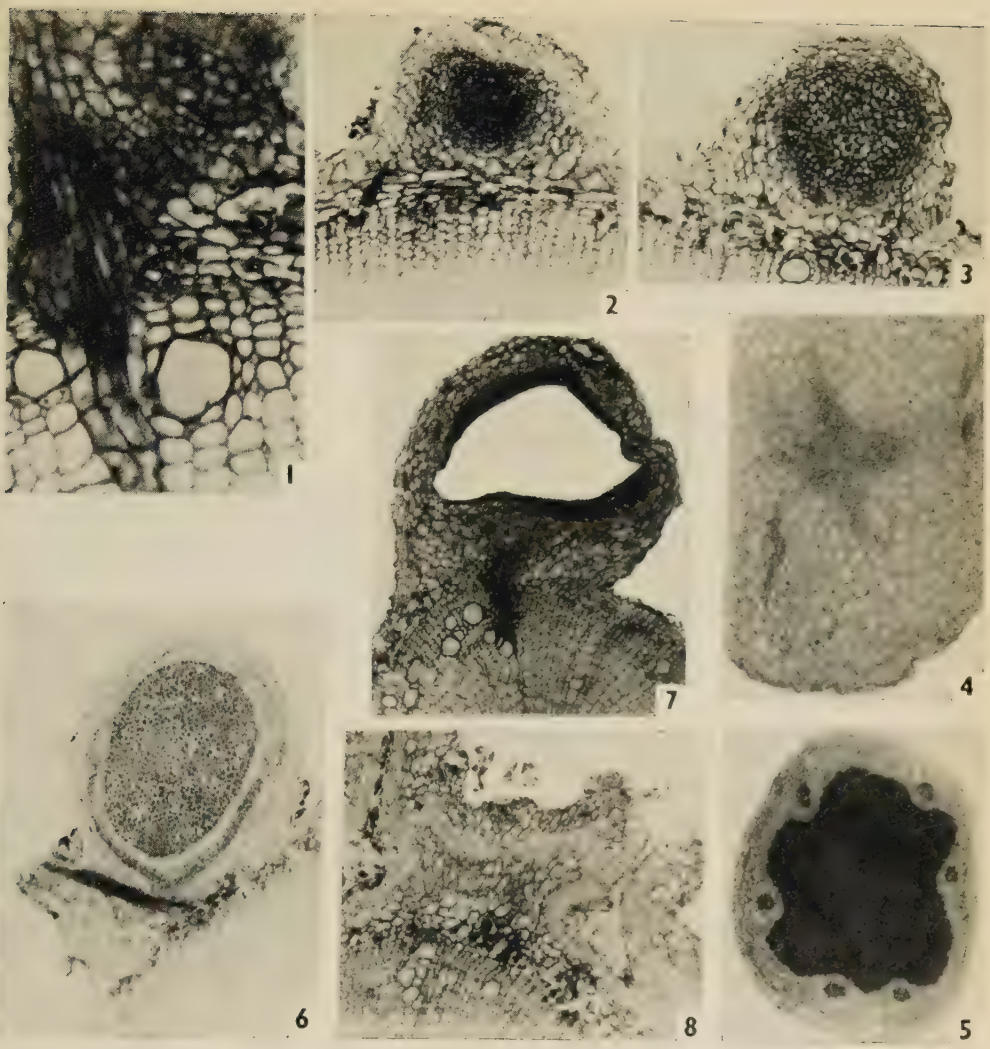
Development of Root Nodules — Root hairs are few and are sloughed off along with the cortex when secondary growth occurs in the root. The nodules arise from pericyclic cells (Figs. 3, 15) in close proximity to the place of emergence of the lateral roots (Figs. 1, 6) and it seems probable that the ruptured tissue in this region constitutes the avenue of infection for the bacteria. No " infection thread " was visible although it is reported in several legumes (Allen & Allen, 1950a) and also occurs in *Cajanus indicus* on which I have made some unpublished observations. Nor were any curled root hairs observed although these were distinct in my preparations of another legume, *Crotalaria juncea*. However, damaged, enlarged parenchymatous cells adjacent to the immature nodular tissue were sometimes found to contain minute irregularly shaped rhizobia.

More advanced stages in nodule formation are seen in Figs. 2, 3. Gradually there is an enlargement and vacuolation of the meristematic cells comprising the nodule.

Development of Stem Nodules — Lateral roots as well as nodules occur abundantly on the stem but only on its lower part which has remained submerged under water for some time. Functional nodules are greenish and have a red interior due to the presence of leghaemoglobin, while the pale ineffective nodules are smaller and devoid of leghaemoglobin. Superficially stem nodules are very similar to those borne on the roots. Like the root nodules they arise near the place of emergence of rootlets (Fig. 16), but have an exogenous origin (Fig. 10). There is no indication of bacteria entering through the lenticels. The youngest stage observed in the development of a stem nodule showed a spherical mass of meristematic cells (Figs. 10, 11) having dense cytoplasm and a large nucleus.

Structure of Mature Nodules — In both roots and stems the mature nodule differentiates into an inner bacteroid and an outer cortical region (Figs. 6, 12). Its cells are smaller than the adjacent parenchymatous cells of the cortex (in the stem) or the pericycle (in the root). Cross-sections of the nodule cut in a plane parallel to the surface of the stem or root show a ring of vascular bundles in the inner part of the nodule cortex. There is no localized meristem; the growth of nodule takes place only by divisions of the infected cells. It does not break through the host tissue but is covered by a number of layers of cells including the periderm (Figs. 6, 12). Stained smears of the bacteroid area showed typical Gram negative rhizobia. The nucleus with one or more nucleoli is situated in the centre of the cells as has already been observed in *Wistaria sinensis* (Jimbo, 1927). As the bacteria multiply within the host cells the vacuoles gradually disappear. In some nodules, binucleate cells were found in the bacteroid region. In the pigmented nodules the bacteria were spherical with occasional rod forms, but in the yellow nodules only rods were present.

Vascular connections between the primary stele and the nodule appear soon after the first few cell divisions have taken place in the nodule after the invasion of bacteria. A single vascular strand enters the nodule and branches to give rise to 6-8 traces (Figs. 4, 5). There is no anastomosing of the branches at the apex of the nodule as reported in *Vigna sinensis* and *Soja man* (Bieberdorf, 1938). In

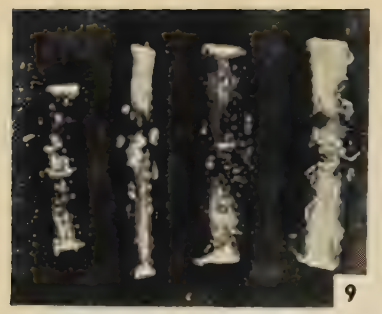


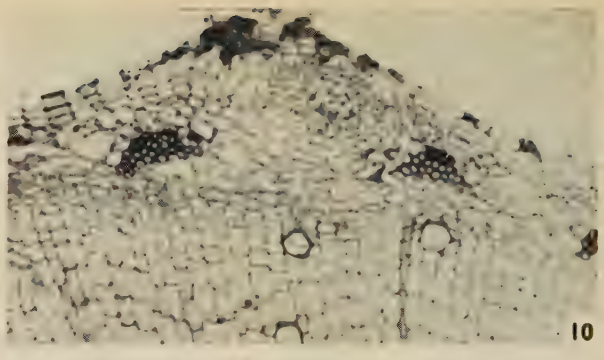
FIGS. 1-8 — Root nodules. Fig. 1. Meristematic nodular tissue and rootlet strand enlarged from portion labelled A in Fig. 17. × 194. Figs. 2, 3. Larger immature nodules. × 79; × 81. Fig. 4. T.S. through basal part of the nodule. × 14·6. Fig. 5. T.S. nodule showing six vascular bundles in the cortex. × 32. Fig. 6. T.S. part of the root passing through mature nodule. × 51. Fig. 7. Hollow nodule. × 58. Fig. 8. T.S. root after the nodule has sloughed off. × 59.

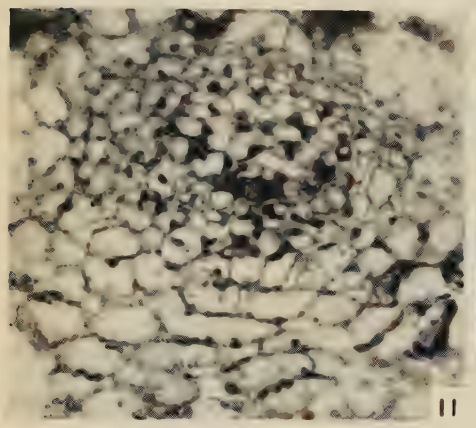
Pisum sativum (Bond, 1948) and *Crotalaria juncea* (Arora, unpublished) where several strands enter the nodule, they may arise at different levels and are traceable to more than one protoxylem point of the root stele. Maturation of the vascular tissues progresses from the base of the nodule to the outside. The xylem elements and phloem elements are arranged collaterally and are surrounded by a typical endodermis with Caspary bands (Fig. 20).

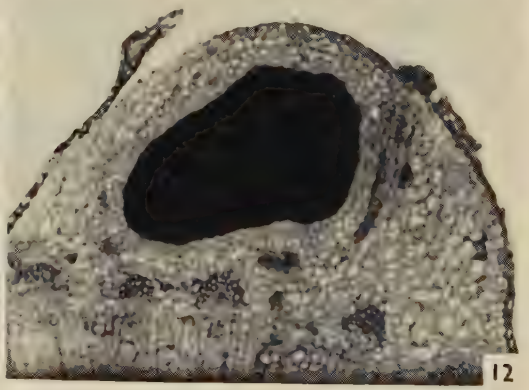
DEGENERATION OF NODULES — Unlike *Sesbania grandiflora* and *Wistaria sinensis* the nodules of *Aeschynomene* are short-lived[2]. Degeneration of the nodule is usually accompanied by the thickening

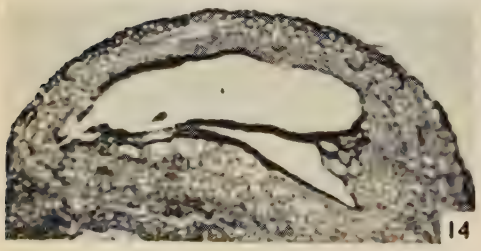
2. At Delhi, plants of *Aeschynomene* come up in July, immediately after the commencement of the rains. They mature and die in Oct.-Nov.





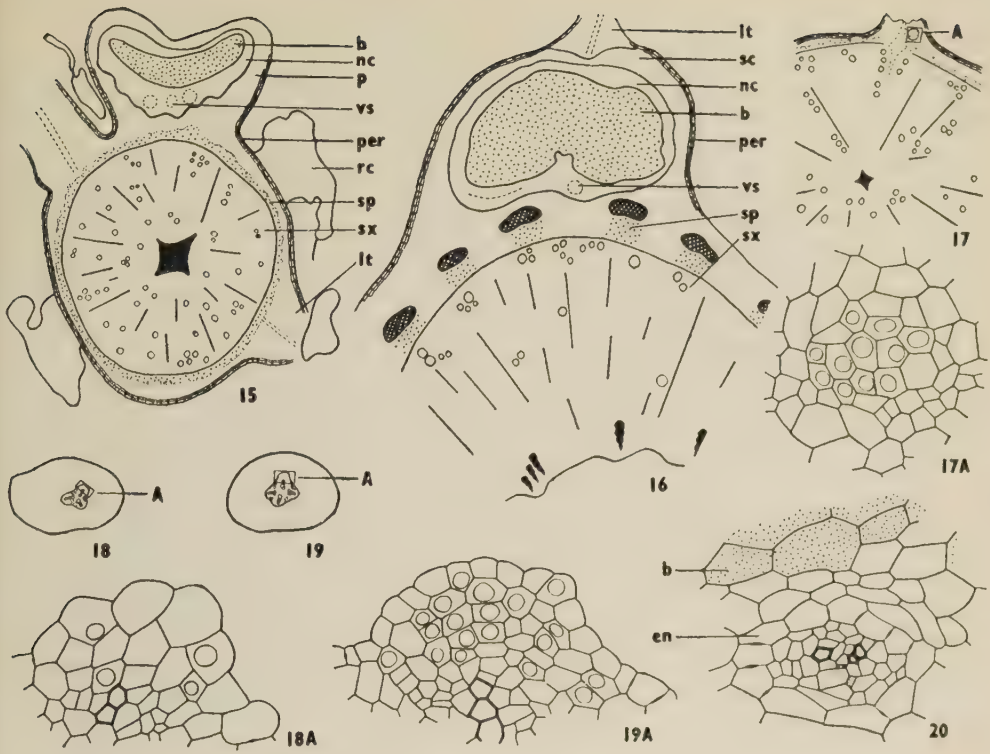






FIGS. 9-14 — Stem nodules. Fig. 9. Pieces of stems showing well-developed nodules. ×1. Fig. 10. T.S. stem with nodular growth in the cortex. × 37·5. Fig. 11. Part of same magnified to show nodular tissue. × 308. Fig. 12. L.S. mature nodule. × 96·5. Fig. 13. Degenerating nodule with a layer of suberized cells forming at the base. 48. Fig. 14. Hollow nodule. × 43.

of a layer of cells at the base of the nodule (Fig. 13). The nuclei of the host cells disintegrate followed by a dissolution of the cell walls in the centre of the bacteroid area and loss of staining capacity by the bacteria. The contents of the nodule exude out from splits in the covering layers. Eventually it is sloughed off and the suberized cell layer forms a protective seal (Fig. 8).



FIGS. 15-20. (*b*, bacteroid region; *en*, endodermis; *lt*, lateral root; *nc*, nodule cortex; *p*, pericycle; *per*, periderm; *rc*, root cortex; *sc*, stem cortex; *sp*, secondary phloem; *sx*, secondary xylem; *vs*, vascular strand). Fig. 15. T.S. root with mature nodule; note exfoliating cortex. × 35·5. Fig. 16. T.S. part of stem showing mature nodule. × 35·5. Fig. 17. T.S. root at the level of young developing nodule and rootlet; part A shown enlarged in Fig. 17A. × 23; × 352. Figs. 18, 19. Diagrams of cross-sections of roots showing developing rootlets. × 23. Figs. 18A, 19A. Same, portions marked A shown more highly magnified. × 352. Fig. 20. Vascular bundle. × 352.

Figs. 7 and 14 show old nodules with a large hollow cavity in the centre, but the cortex and vascular traces are still intact. In some nodules there were surface cracks through which the bacteria might be exuded to the soil.

Discussion

As a rule the bacterial nodules on roots have an exogenous origin while lateral roots originate endogenously. Perhaps the only recorded exception is *Arachis hypogea* (Allen & Allen, 1940) in which the nodules as well as lateral roots arise in the pericycle and later extrude through the cortex. In *Aeschynomene indica* also the root nodules arise in the pericycle and are therefore endogenous but were never seen to emerge out of the periderm. In contrast, the stem nodules are exogenous and arise in the cortex; and the covering periderm develops from the subepidermal layers of the cortex.

As opposed to the condition found in *Medicago sativa* (Thornton, 1930), *Pisum sativum* (Bond, 1948), *Sesbania grandiflora* (Harris, Allen & Allen, 1949), and *Soja man* (Bieberdorf, 1938), in *Aeschynomene* the rhizobia do not enter the host through the root hairs but through ruptured cortical tissues. The course of nodule development in *Aeschynomene* is fundamentally the same as that in *Arachis hypogea* (Allen & Allen, 1940), but the former differs in having no localized meristem

and in the fact that all the cells of the bacteroid area are infected and non-vacuolated.

Peirce (1902) describes the tubercles and lateral roots of *Medicago denticulata* as morphologically similar. The tubercles, he says, have the same form as rootlets; and in both cases the apical meristem consists of embryonic, meristematic cells. In *Aeschynomene*, however, the primordia of rootlets and nodules can be distinguished even after the first few divisions of the pericyclic cells (Figs. 17-19). There is no localized meristem in the nodules of *Aeschynomene* nor any nodule endodermis but a separate endodermis around each bundle, while a lateral root has a single endodermis enclosing the stele. The heavily lignified sclereids present in the outer part of the nodule cortex of *Sesbania grandiflora* (Harris, Allen & Allen, 1949), *Wistaria sinensis* (Jimbo, 1927) and *Soja man* (Bieberdorf, 1938) are also absent in *Aeschynomene*.

In *Aeschynomene* the suberized layer of cells formed at the base of the nodule does not cut off the main vascular supply to it and the vascular tissues do not become indistinct as reported in *Arachis hypogea* (Allen & Allen, 1940). In *Sesbania* (Harris, Allen & Allen, 1949) there is no suberized thickening at the base of the nodule, the first indication of degeneration being the mottled appearance of the nodule.

Summary

1. *Aeschynomene indica* L. has well-developed nodules on the roots as well as the stem.

2. Nodules arise close to the place of emergence of the lateral rootlets, and the ruptured tissue in this region constitutes the path of infection. Infection does not seem to take place through the root hairs.

3. Root nodules are endogenous and arise in the pericycle while stem nodules originate in the cortex.

4. There is no localized meristem, and cell divisions are widespread in the infected cells.

5. The nodule is differentiated into an infected bacteroid region and an uninfected portion.

6. A single vascular strand differentiates at the base of the nodule and gives off 6-8 branches which taper unequally to the apex.

7. The vascular tissues of the nodule mature from the base outwards. The xylem and phloem are arranged collaterally. An endodermis with typical Caspary bands surrounds each vascular strand.

8. Degeneration of the nodule is accompanied by the formation of a layer of suberized cells at its base. When the nodule is sloughed off, the suberized layer forms a protective seal.

I wish to express my sincere gratitude to Prof. P. Maheshwari under whose guidance this work has been carried out.

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FLORAL MORPHOLOGY AND EMBRYOLOGY OF *LIPPIA NODIFLORA* RICH.

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Introduction

The earliest embryological contribution on the family Verbenaceae is by Hofmeister (1858)[1] and deals with the development of the endosperm haustoria in *Verbena officinalis*. Warming (1873)[1] and Jönsson (1879-80)[1] also worked on the same species. Koorders (1896)[1] studied the morphology, physiology and embryology of *Tectona grandis* and reported a cellular endosperm having large " absorbing bladders " at the micropylar end.

Among recent authors, mention may be made of Junell (1934) and Patermann (1935) who made an extensive study of the floral morphology and embryology of several members of the family. Souèges (1935a, b) and Crété (1942) have made detailed observations on the development of the endosperm and embryo of *Verbena officinalis* and *Lantana camara*. On *Lippia* the chief work is by Schwencke (1931) and Pal (1951). Schwencke (1931) studied three species, *L. chamaedrifolia*, *L. citriodora* and *L. lycioides*. In the first named, he noted the formation of " dwarf " pollen under the influence of cold and a frequent degeneration of two-nucleate pollen grains and of the egg apparatus. In *L. citriodora* there was a prompt degeneration of uninucleate pollen grains. In both *L. citriodora* and *L. lycioides* degenerations in the ovule usually take place before the mature embryo sac stage. Pal (1951) worked on the female gametophyte, embryo development and endosperm haustoria of *Lippia nodiflora*, *Tectona grandis* and *Vitex negundo*. He found that in all cases the chalazal haustorium is unicellular and binucleate and is differentiated directly after the first division of the primary endosperm nucleus. The micropylar haustorium of *Lippia nodiflora* is multicellular and the embryo is of the " Solanad type ".

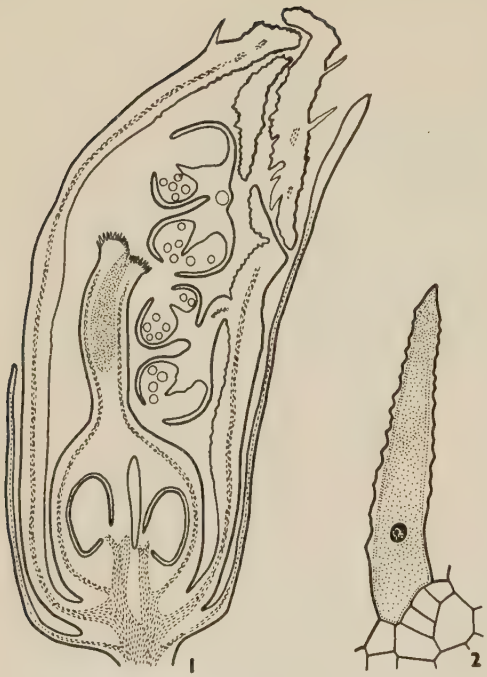
1. Quoted from Schnarf (1931).

Material and Methods

There are about 60 species of the genus *Lippia* occurring chiefly in tropical America. Of these *L. nodiflora* and *L. citriodora* extend to India. The former is found throughout India and is very widely used as a febrifuge and as a diuretic. It flowers during the rainy season and is common in moist places. Buds, flowers, young inflorescences and fruits of *Lippia nodiflora* were collected from the banks of Jumna and fixed in formalin-acetic-alcohol. The young inflorescences were trimmed on two parallel sides to facilitate the penetration of the fixing fluid. Developing fruits were treated with 10 per cent hydrofluoric acid in 70 per cent alcohol for 20-30 days to soften the seed coat and the pericarp. The customary methods of dehydration and embedding were followed. Sections were cut at a thickness of 6-10 μ for younger and 12-16 μ for older stages. Considerable difficulty was experienced in sectioning due to the presence of glands and hairs on the floral envelopes and also on account of the hard endocarp. The sections were stained in Heidenhain's iron alum hematoxylin or safranin-fast green. A very dilute solution of fast green in alcohol was sometimes used after hematoxylin as a counterstain.

Observations

Morphology of the Flower — The flowers are sessile and arranged in dense, globose, peduncled, axillary heads which



FIGS. 1, 2 — Floral morphology. Fig. 1. L.S. young flower. × 59. Fig. 2. Epidermal hair from bract. × 38.

elongate at maturity to form oblong spikes. Each flower is subtended by a large boat-shaped bract. The floral envelopes and bracts are both covered by unicellular and multicellular hairs and glands of various kinds. The hairs may show warty thickenings on their walls (Fig. 2).

The sequence of floral development is calyx androecium, corolla and gynaecium. The primordia of the two carpels curve inwards, meet and fuse to give rise to the style and stigma. The cells of the central portion of the style elongate and become free from each other at an early stage of development so as to give rise to large intercellular spaces (Fig. 1). The placentae arise from the basal portions of the carpels.

DEVELOPMENT OF THE ANTHER — A cross-section of a very young anther shows a mass of homogeneous meristematic cells surrounded by the epidermis. Later, it becomes slightly oval and two-lobed. The hypodermal archesporium differentiates in each lobe (Fig. 3) and is recognizable by the larger size, denser cytoplasm and more conspicuous nuclei of its cells. As the anther grows further and becomes four-lobed, sterilization starts in the middle of each lobe thereby leading to the division of the archesporial group on each side into two portions (Fig. 4). Misra (1939) and Kanda (1920) report that in *Lippia nodiflora* and *Verbena* the archesporium appears at the four-lobed stage but it is probable that they missed the younger stages in the development of the anther.

MICROSPOROGENESIS — The archesporium comprises a plate of 15-16 cells in each lobe (Fig. 5) which is 8-10 cells deep in longitudinal section. The outermost layer of archesporial cells divides periclinally to cut off the primary parietal layer. This divides giving rise to the endothecium and an inner layer (Fig. 6). The latter divides once more to produce the middle layer and the tapetum (Fig. 7).

The middle layer begins to degenerate soon after the formation of the microspores (Fig. 8).

The tapetum is of the glandular type and most of the cells become binucleate by mitotic divisions (Fig. 7). Occasionally trinucleate and tetranucleate cells may also be observed. *Verbena* (Schwencke, 1931), *Callicarpa* (Patermann, 1935) and *Premna* (Patermann, 1935)

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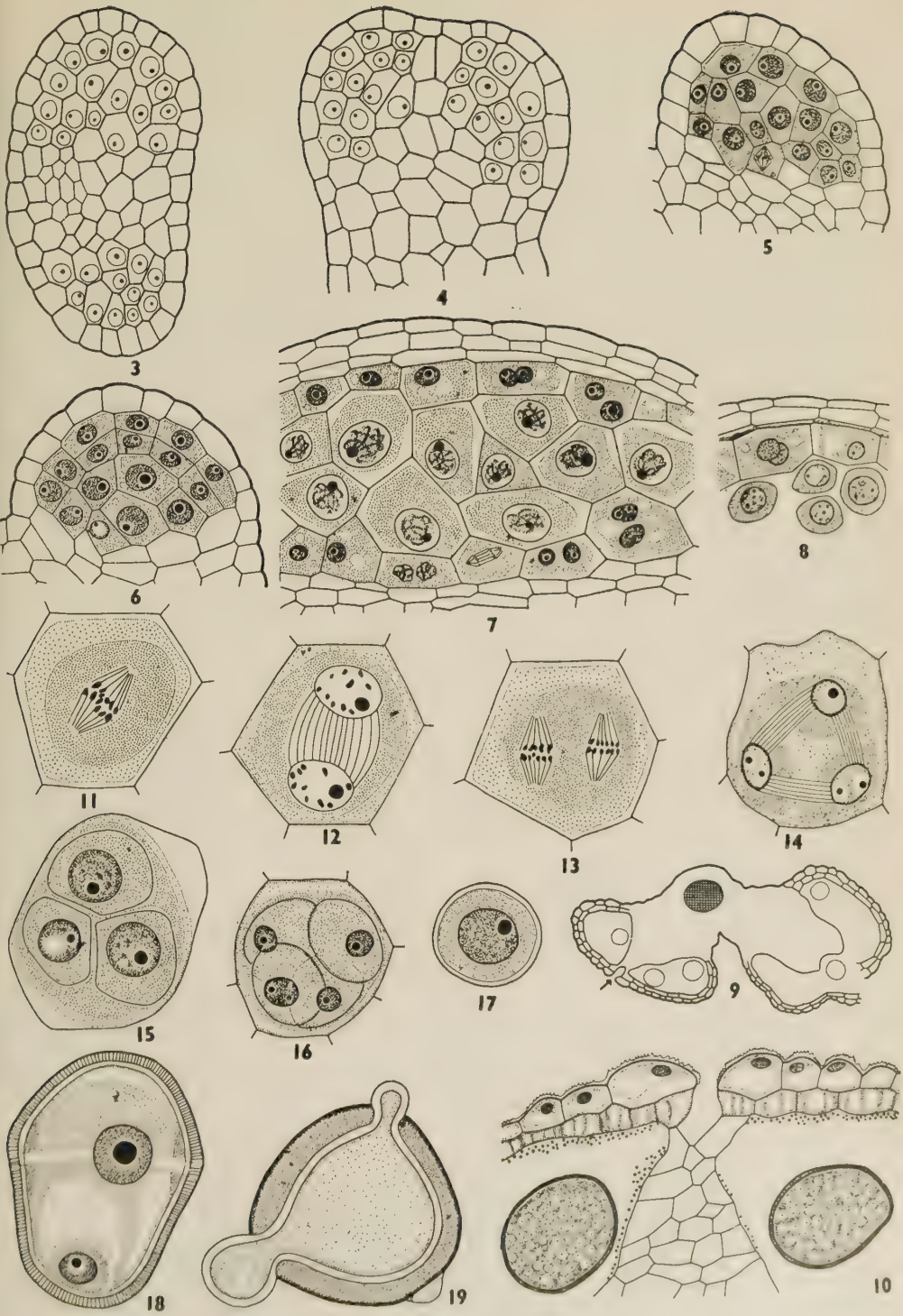
FIGS. 3-19 — Microsporogenesis and male gametophyte. Fig. 3. T.S. anther showing differentiation of archesporium at two-lobed stage. × 668·5. Fig. 4. Anther lobe with two groups of archesporial cells separated by a sterile plate. × 668·5. Figs. 5, 6. Single anther lobe showing sporogenous tissue and primary parietal layer. × 668·5. Fig. 7. Part of anther lobe showing two-nucleate tapetal cells and microspore mother cells in early prophase. × 668·5. Figs. 8, 10. Anther wall at microspore and mature pollen grain stage. × 668·5. Fig. 9. T.S. anther at the time of dehiscence. × 141. Figs. 11, 12. Microspore mother cells in Meiosis I. × 1337. Fig. 13. Meiosis II. × 1337. Fig. 14. Cytokinesis, the equatorial region between the daughter nuclei is vacuolated. × 1337. Figs. 15, 16. Tetrahedral and decussate tetrads. × 1337. Figs. 17, 18. Uni- and bicelled pollen grains. × 1337. Fig. 19. Whole mount of pollen grain to show germ pores. × 1003.



TEXT-FIGS. 3-19.

also show 4-nucleate tapetal cells. Some of the nuclei are amoeboid and contain more than one nucleolus (Fig. 7) suggesting their polyploid nature. There is no periplasmodium formation. Only in *Lantana*, according to Patermann (1935), individual cells and cell complexes wander in between the pollen grains although without forming a periplasmodium. The tapetum shows signs of degeneration when the pollen grains are one-celled.

The endothecial cells (Figs. 9, 10) elongate tangentially and their walls become greatly thickened but the characteristic fibrous thickenings are noticeable only when the pollen grains are about to be shed. At this stage the walls of the epidermal cells bulge out and the cuticle shows peg-like projections.

As the tapetum is used up, minute oil globules appear on its inner side and become conspicuous during the maturation of the pollen grains (Fig. 10). After the disappearance of the tapetum, these globules are seen against the inner wall of the endothecium. They stain brownish-yellow with hematoxylin and red with safranin. Such globules have not been reported previously in the Verbenaceae. Their exact nature and function is not understood but it is probable that they may contribute to the formation of the exine (see Maheshwari, 1950).

The primary sporogenous cells divide once or twice to give rise to the microspore mother cells (Fig. 7). As they prepare for meiosis, their protoplasts recede from the mother walls which remain in contact with each other (Fig. 7). A special mucilaginous wall appears between the protoplast and the original wall (Figs. 11-13). Wall formation is of the simultaneous type (Figs. 11-13). In Meiosis II, according to Kanda (1920) and Schwencke (1931), the spindles lie crosswise. In the plants investigated by Patermann (1935) as well as in *Lippia* studied here, the spindles usually lie parallel to each other (Fig. 13). Cytokinesis takes place by centripetally advancing constriction furrows which meet in the centre and divide the mother cell into four parts. The microspore tetrads may be tetrahedral (Fig. 15), decussate (Fig. 16) or isobilateral.

In some cases, the three layers of the anther wall, i.e. the epidermis, the endothecium and the middle layer were well developed but the rest of the anther was empty. Since the walls of the tapetal and sporogenous cells were still distinguishable, it appears that the nuclei and cytoplasm abort at an early stage. The actual course of degeneration could not be traced, however.

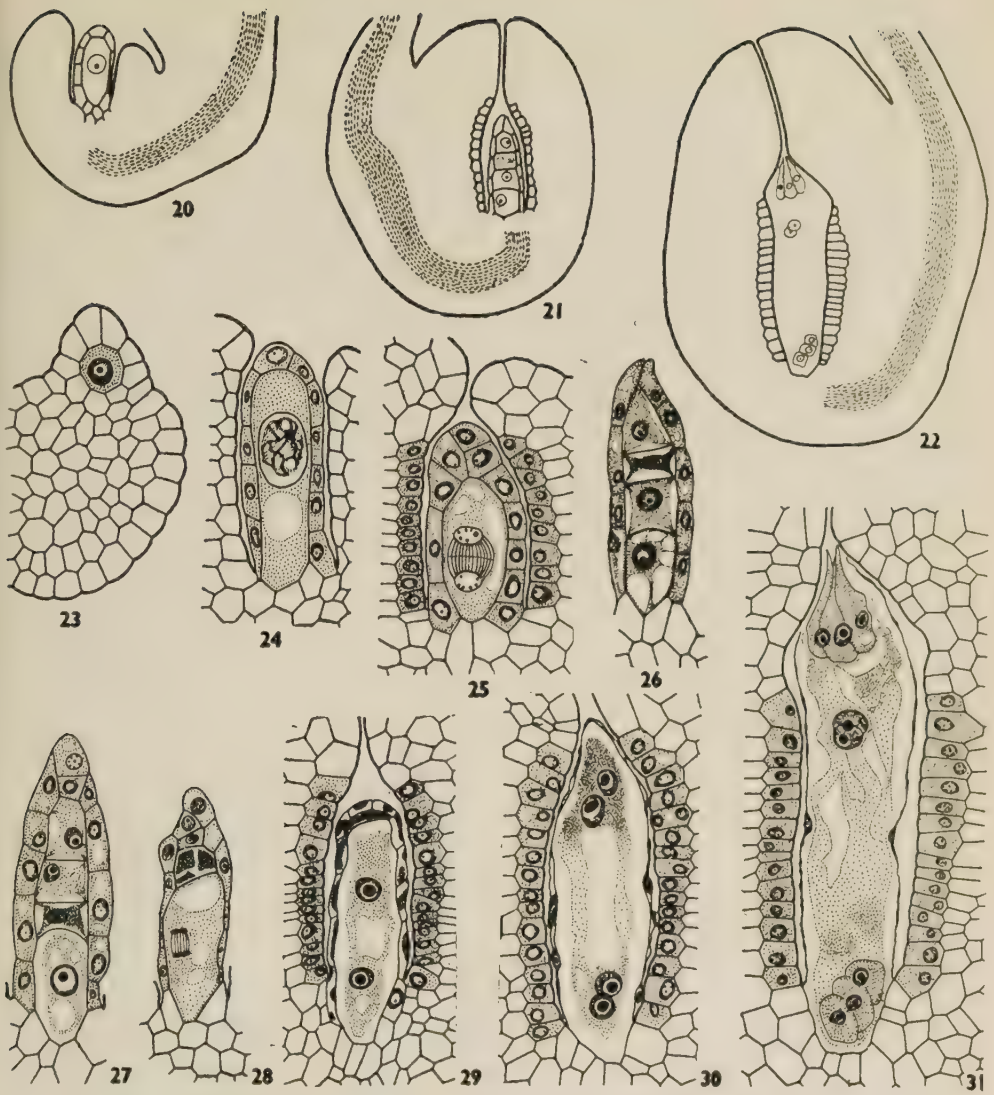
Male Gametophyte — A newly formed microspore has dense cytoplasm with a centrally situated nucleus. Its wall becomes differentiated into a thick exine and a thin intine (Fig. 17). Kanda (1920) has reported the occasional occurrence of starch grains in the three species of *Verbena* studied by him. There are generally three germ pores (Fig. 19).

As the pollen grain enlarges, a large vacuole appears in the cytoplasm and pushes the nucleus to one side. The first division produces a small generative and a larger tube cell (Fig. 18) separated by an ephemeral membrane. After the dissolution of the separating wall, the lenticular generative cell moves up near the tube nucleus. The pollen grains are shed at the two-celled stage.

Ovule — The unitegmic, tenuinucellate ovule becomes anatropous by the time the megaspore mother cell is formed (Fig. 20). The nucellar epidermis is consumed by the enlarging megaspore and the innermost layer of the integument differentiates into an endothelium (Figs. 29, 30). The vascular trace entering the funiculus terminates at the chalaza (Figs. 20-22).

Megasporogenesis — A hypodermal archesporial cell differentiates in the young nucellus (Fig. 23) at the time when the microspore mother cells are in the resting condition. In *Premna integrifolia* (Patermann, 1935) and rarely in *Lippia nodiflora* (Pal, 1951), a two-celled archesporium has been recorded. The archesporial cell functions directly as the megaspore mother cell. Generally the nucellus remains one-layered but sometimes one or two cells of the nucellar epidermis may divide periclinally (Fig 27).

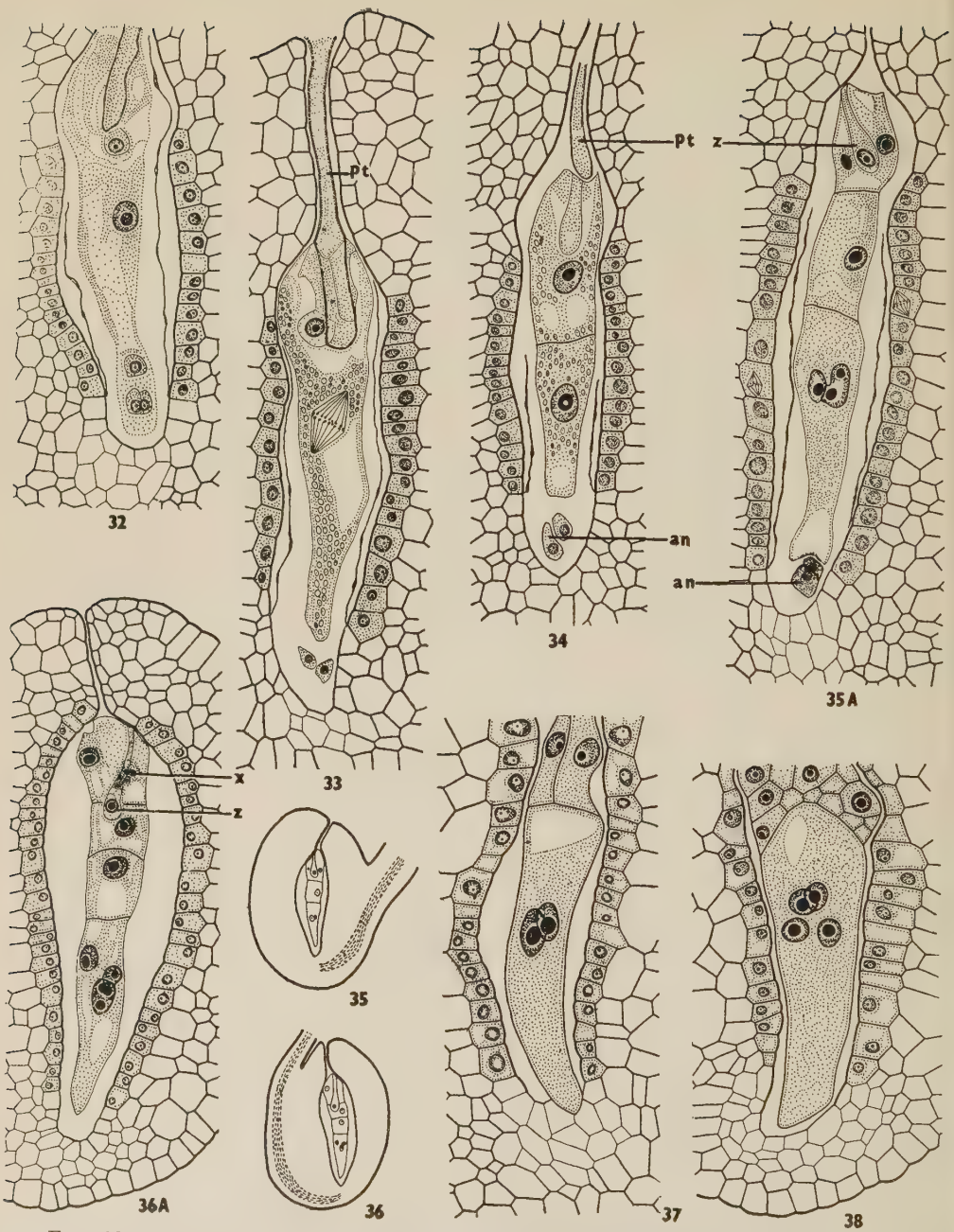
The megaspore mother cell (Figs. 24, 25) enlarges considerably and divides to give rise to a linear row of four mega-



FIGS. 20-31 — Megasporogenesis and female gametophyte. Figs. 20-22. L.S. ovules at progressively older stages. × 284. Fig. 23. Hypodermal archesporial cell. × 662. Figs. 24, 25. Megaspore mother cells; the endothelium is differentiating. × 662. Figs. 26-28. Tetrads of megaspores. × 662. Figs. 29,30. Two and four-nucleate embryo sacs. × 662. Fig. 31. Organized embryo sac. × 662.

spores (Fig. 26). Only in *Verbena officinalis*, according to Schwencke (1931), there is a T-shaped arrangement of the megaspores. Of the four cells the upper three degenerate while the chalazal (Fig. 27) develops into an embryo sac. Occasionally the micropylar megaspore remains healthy for some time while the second and third megaspores degenerate earlier. *Citharexylum ilicifolium* (Patermann, 1935) is an exception as here the third megaspore gives rise to the embryo sac.

FEMALE GAMETOPHYTE — The functioning megaspore elongates and becomes vacuolated and its nucleus comes to lie



FIGS. 32-38. Development of endosperm (*an*, antipodals; *pt*, pollen tube; *x*, x-bodies; *z*, zygote). Fig. 32. Fertilized embryo sac showing zygote, primary endosperm nucleus and three antipodal cells. × 607. Figs. 33, 34. First division of primary endosperm nucleus. × 607. Fig. 35. Ovule showing first transverse division in micropylar chamber. × 95. Fig. 35A. Embryo sac of Fig. 35 enlarged to show first transverse division in the micropylar chamber; the chalazal haustorium is two-nucleate; remnants of antipodals and nucellus are still distinguishable; endothelial cells are dividing. × 607. Fig. 36. L.S. ovule showing second division in micropylar chamber. × 95. Fig. 36A. Embryo sac of Fig. 36 shown enlarged; the chalazal haustorium shows three nuclei; note x-bodies around the undivided zygote. × 390. Figs. 37, 38. Chalazal haustorium with 2 and 4 nuclei respectively. × 607.

more or less in the centre (Fig. 27). It soon divides to give rise to the two-, four- and eight-nucleate stages (Figs. 28, 29, 30, 31).

The cells of the endothelium become radially elongated and have prominent nuclei and dense cytoplasm. They show their maximum activity just after fertilization and undergo divisions in keeping with the elongation of the embryo sac. However, as the endosperm develops, the nutritional function of these cells slows down. Their nuclei gradually degenerate and the cells become vacuolated. No tapetum is organized in the micropylar part of the integument (Fig. 31) and the cells in this region become filled with starch grains. In *Vitex negundo* (Pal, 1951) the tapetum differentiates only around the middle portion of the embryo sac.

The mature embryo sac (Fig. 31) has a broader micropylar end which tapers downward. Junell (1934) has described different forms of embryo sacs in the family. In *Clerodendron* (Viticoideae) the lower part is enlarged like a sac; in *Petraea* (Verbenoideae), *Pityrodia* (Chloanthoideae) and *Congea* (Symphoremoideae) the embryo sac protrudes beyond the micropyle and lies partly free in the loculus. The egg apparatus consists as usual of an egg and two synergids (Fig. 31). The latter are elongated and pear-shaped and have a vacuole in the basal end. They have prominent hooks and pointed beaks as also reported by Misra (1939) in *Clerodendron phlomidis* and *Caryopteris wallichiana*. The flask-shaped egg has a vacuole towards its micropylar region and protrudes below the synergids. The three antipodal cells (Fig. 31) are situated in the narrow chalazal end and two of them lie below the third to form a pyramidal structure. The lower two show some elongation but all degenerate soon after the first few divisions of the primary endosperm nucleus. In *Lippia nodiflora* the polar nuclei (Fig. 32) fuse after the entry of the pollen tube but in *Cornutia* and *Clerodendron* (Junell, 1934) the fusion occurs before fertilization. Starch is abundant in the general cytoplasm of the embryo sac.

Pollination and Fertilization — Self-pollination may occur since the pollen and the embryo sac mature at about the same time but cross-pollination seems to be more common.

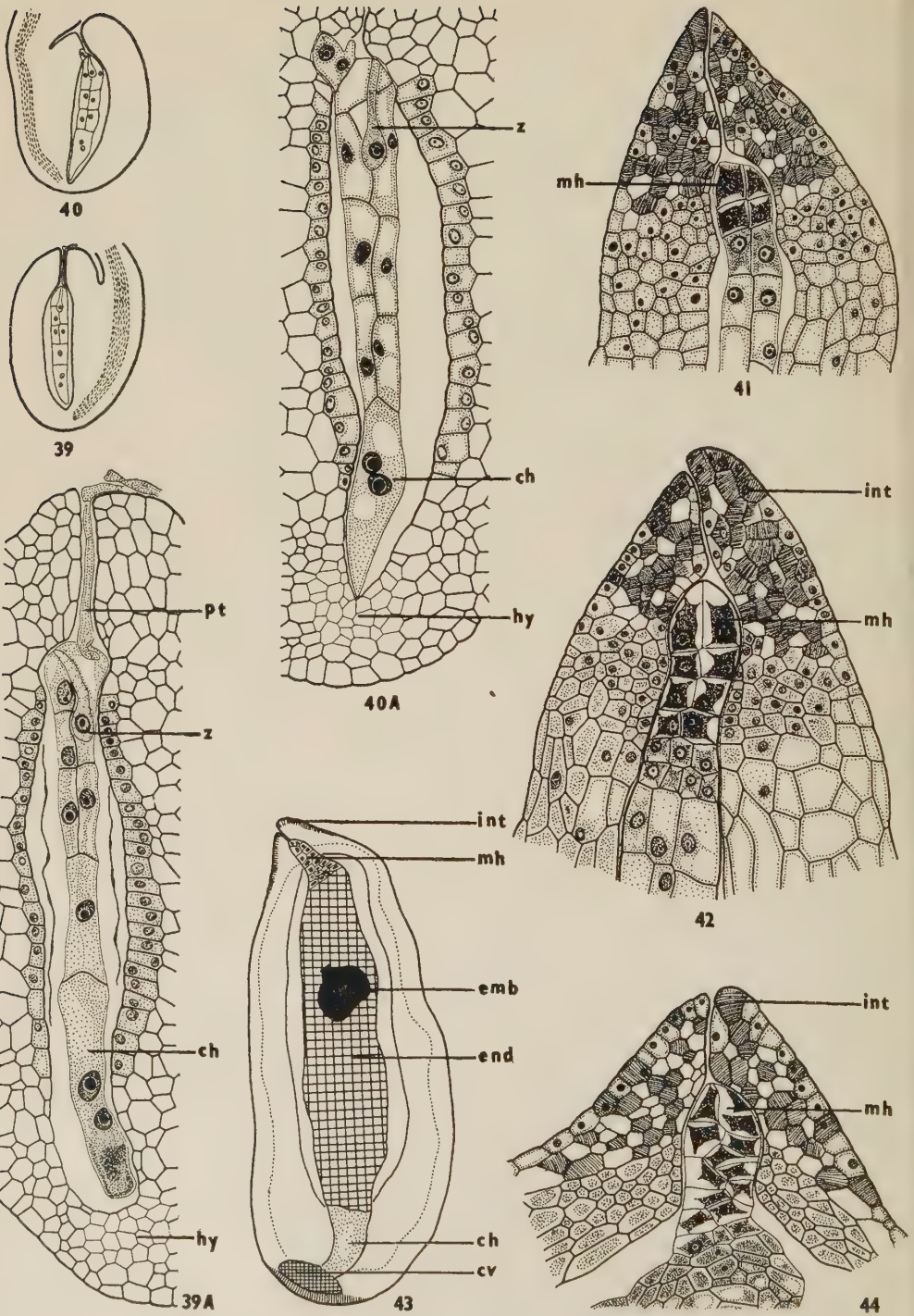
The hollow style is often crowded with pollen tubes. On reaching the ovary the pollen tube passes through the micropyle and as it enters the embryo sac, it demolishes one of the synergids. Double fertilization was not actually observed but may be presumed to occur normally. It has been reported by Schnarf (1925) in *Verbena angustifolia* and by Junell (1934) in *Verbena* and *Amethystea*.

Endosperm — Division of the primary endosperm nucleus (Figs. 32, 33) is followed by a transverse wall giving rise to the primary micropylar and the primary chalazal chambers (Fig. 34). The nucleus of the latter divides without any wall formation and the binucleate cell functions as the chalazal haustorium (Figs. 35, 35A, 37) which elongates towards the conducting tissue (Fig. 43). Occasionally the two nuclei divide once more resulting in a four-nucleate haustorium (Fig. 38). The nuclei are situated near the centre and a vacuole frequently appears in the apical part (Fig. 37). Some of the cells below the haustorium are thin-walled with denser contents and form a weak hypostase (Figs. 32-38, 39A, 40A).

According to Pal (1951), " the nucleus of the primary micropylar chamber divides and a longitudinal wall is formed in between the two resulting nuclei. Then, both the cells divide by successive transverse walls and ultimately five tiers of cells (including the chalazal haustorium) are formed ".

In my material, however, the first two divisions in the micropylar chamber are transverse (Figs. 35, 36) and it is after this that longitudinal walls (Figs. 39, 40) are laid down. If Pal's observations are correct, this would indicate a considerable range of variability in this species. According to Souèges (1935) in *Verbena officinalis* also, the first wall in the micropylar chamber may be either longitudinal or transverse.

The cells of the uppermost tier differentiate into the micropylar haustorium. This functions for a short time only, after which the cells below it and then those next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FIGS. 39-44.

below function similarly and behave as haustoria (Figs. 41, 42). In this way, many of the endosperm cells take part in the formation of the micropylar haustorium. Similar development has also been observed in *Amethystea* (Junell, 1934) and in *Tectona* (Pal, 1951).

The micropylar haustorium forms a short narrow structure (Fig. 43). According to Pal (1951), the cells of the micropylar haustorium round up and ultimately become free from the endosperm cells. I am unable to confirm this. Using fast green as a counterstain after iron hematoxylin, I could see quite distinctly that the haustorial cells maintain their connection with the endosperm proper.

Due to the activity of the haustorial cells, the cells of the integument at the micropylar end are emptied of their contents leaving only its outer epidermis and the cells at the tip intact. The walls of the latter become reticulately thickened (Figs. 41, 42, 44).

As the proembryo advances beyond the globular stage, the micropylar haustorium becomes non-functional. The chalazal haustorium persists somewhat longer and degenerates only after the heart-shaped stage (Fig. 43).

In later stages, when both the haustoria have degenerated, the peripheral layers of the endosperm become prominent and undergo periclinal divisions to form a cambium-like zone consisting of 5-6 layers of cells with dense cytoplasm and prominent nuclei (Fig. 58). The outer two layers of this zone persist in the mature seed.

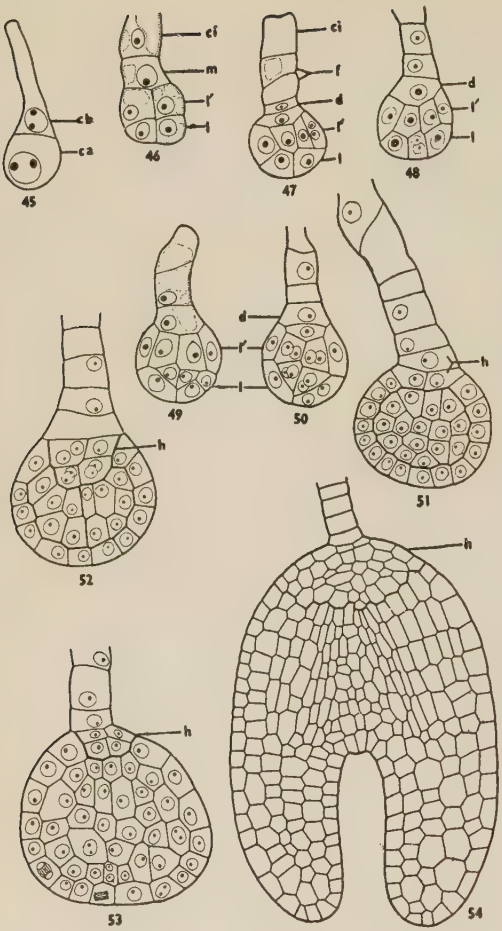
Development of the Embryo — After fertilization the zygote elongates considerably (Figs. 39A, 40A). The first division of the zygote takes place when about 10-12 tiers of endosperm cells have been formed and the haustoria are already well differentiated. The first wall is transverse (Fig. 45) resulting in the terminal cell *ca* and the basal cell *cb*. The latter divides transversely to form the cells *m* and *ci* (Fig. 46). The division of *m* forms the tiers *f* and *d* whereas the cell *ci* remains undivided at this stage (Fig. 47).

The cell *ca* undergoes two divisions at right angles to each other resulting in a quadrant. The next division is transverse so that an octant is formed (Fig. 46). The tiers of the octant may be designated as *l* and *l'*. Periclinal divisions cut off the dermatogen (Figs. 49, 50) which continues to extend by anticlinal divisions (Fig. 51). The differentiation of other histogenic layers follows. The divisions in the axial cells lead to the formation of the periblem and the plerome (Figs. 52, 53). Further divisions in the latter are anticlinal as well as periclinal.

The penultimate cell *d* of the proembryo (Fig. 48) functions directly as the " hypophysis ". It divides transversely at the time of differentiation of the dermatogen to form two daughter cells. Each of these undergoes two further divisions by walls which are oriented at right angles to one another (Figs. 52, 53). The hypophysis forms the initials of the root cortex, root cap and the root epidermis. All the cells next to the penultimate cell function as suspensor. This becomes inconspicuous and degenerates after the differentiation of the cotyledons. The tier *l* gives rise to the cotyledons and stem tip and *l'* to the hypocotyledonary region. The stem tip is very small. The cotyledonary initials arise from the peri-

←—

Figs. 39-44 — Older stages in endosperm development (*ch*, chalazal haustorium; *cv*, chalazal vascular trace; *emb*, embryo; *end*, endosperm proper; *hy*, hypostase; *int*, specialized cells of the integument; *mh*, micropylar haustorium; *pt*, pollen tube; *z*, zygote). Fig. 39. L.S. ovule showing longitudinal division in micropylar chamber. × 104. Fig. 39A. Enlarged view of endosperm in Fig. 39. × 422. Fig. 40. L.S. ovule showing older stage. × 104. Fig. 40A. Part of Fig. 40 enlarged; the uppermost tiers form the micropylar haustorium. × 422. Fig. 41. Upper two tiers of cells in process of degeneration; third tier shows signs of haustorial activity; note thickenings in the integumentary cells in the micropylar region. × 422. Fig. 42. Micropylar haustorium at the stage of proembryo shown in Fig. 49. × 422. Fig. 43. L.S. ovule showing multicellular micropylar haustorium and degenerated chalazal haustorium. × 104. Fig. 44. Micropylar haustorium at the stage of embryo shown in Fig. 53. × 422.



FIGS. 45-54 — Stages in development of the embryo; for explanation see text. Figs. 45-53. × 437·5 and Fig. 54. × 281.

pheral region of apical tiers and grow very vigorously to give rise to two large cotyledons (Fig. 54). The embryogeny, therefore, corresponds to the Crucifer type.

SEED — At the mature embryo sac stage the integument (Fig. 55) comprises 6-7 layers of starchy cells. At the proembryo stage its cells become larger and more vacuolate due to the absorption of food material by the outer layers of the endosperm. A few of the outer layers of the integument (Fig. 56) show well developed reticulate thickenings. At the time of differentiation of the cotyledons the integument (Fig. 57) consists of an outer zone of 2-3 layers of cells with well developed reticulate thickenings and an inner zone of 3-4 layers of cells with cutinized walls. The nuclei degenerate and become pressed against the walls. The cells of the epidermal layer as well as those of the inner part of the integument become stretched out tangentially and show cutinized walls.

During early post-fertilization changes, the epidermal cells of the integument (Fig. 43) situated at the extreme chalazal end and those at the extreme micropylar end become markedly different in appearance. They show reticulate thickenings staining deep red with safranin on their radial walls but still possess cytoplasm and nuclei (Fig. 44). Certain shining crystals have been observed in the cells surrounding the chalazal bundle.

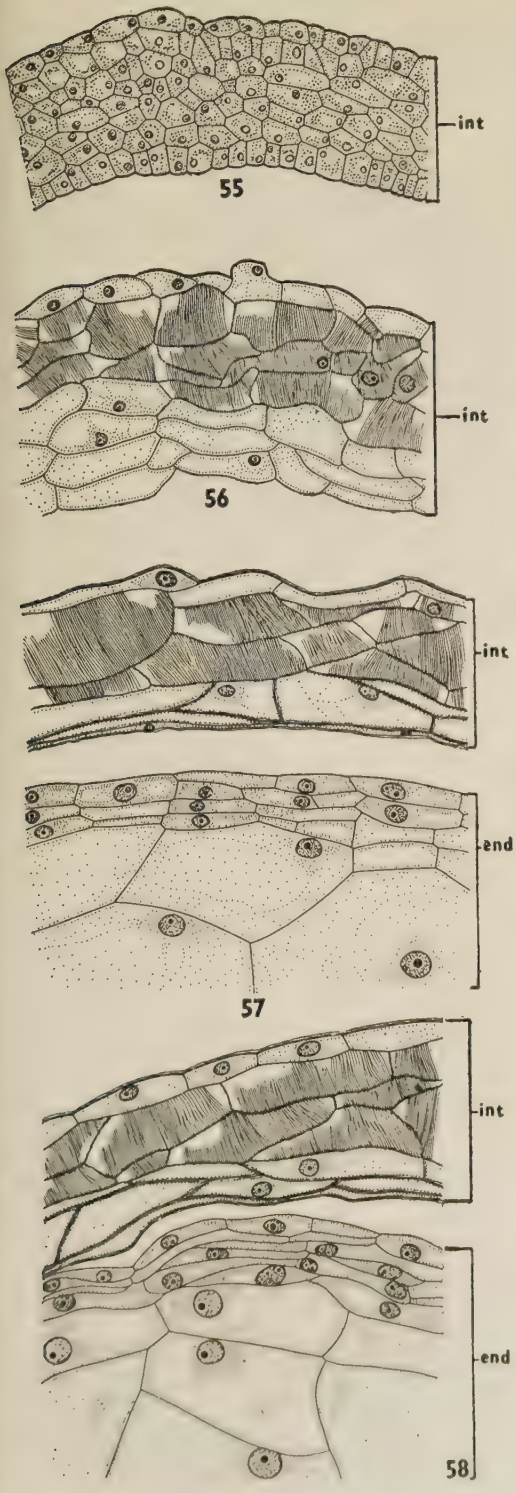
Only the outer one or two layers of the integument persist in a mature seed (Fig. 63). At this stage, the embryo nearly fills the whole space, except for the two persistent layers of endosperm cells which lie directly under the seed coat.

PERICARP — At the time of fertilization the pericarp (Fig. 59) consists of 16-18 layers of parenchymatous cells. Later, at the young globular stage of the proembryo, it differentiates into two regions — an outer of closely compressed cells with small nuclei and an inner of bigger cells with prominent nuclei. Subsequently thickenings are laid down on the walls of the inner part of the pericarp (Fig. 60). Similarly, at a slightly later stage, the cells of the outer region also show pits on their walls (Fig. 60).

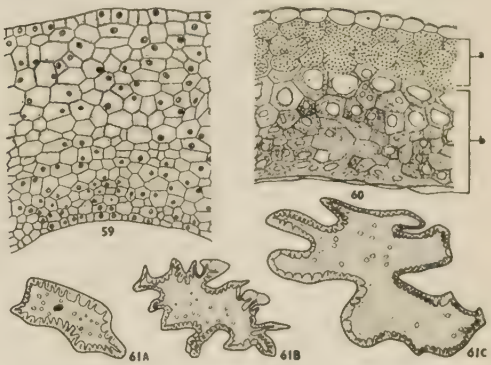
Macerations of the pericarp from the inner region show sclereids of various sizes and patterns and frequently provided with well-developed horns or processes (Figs. 61A, B, C).

Discussion

The family Verbenaceae represents an assemblage of plants showing many common features of floral morphology and embryology. However, several workers have made contradictory statements which require discussion in detail.



As a rule, the pollen grains are shed at the two-celled stage. According to Schnarf (1937), two-nucleate pollen grains occur in *Verbena officinalis, Lippia citriodora, Vitex agnus-castus, Verbena hybrida* and *Clerodendron foetidum.* In *Duranta plumieri* (author's unpublished observations) and *Lippia nodiflora* also the pollen is shed at the two-celled stage. Only in *Stachytarphcta dichotoma,* Junell (1934) reports a three-nucleate condition with two small sperm nuclei. This needs confirmation.



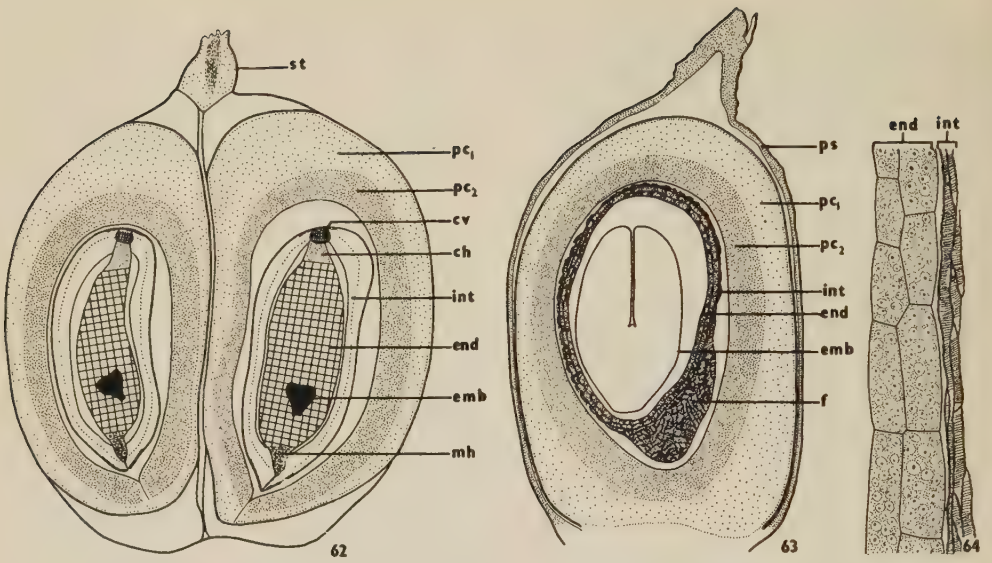
FIGS. 59-61 — Pericarp (*a*, outer region of pericarp with simple pits; *b*, inner region composed of sclereids). Fig. 59. Pericarp at early stage. × 225. Fig. 60. Same, from mature fruit showing outer thin-walled and inner thick-walled region. × 225. Figs. 61A, B, C. Different types of sclereids from inner region of pericarp (from macerated preparation). × 450.

The ovules are unitegmic and tenuinucellate. The nucellus disorganizes before the four-nucleate embryo sac is formed. Kanda (1920) reported that in *Verbena,* a nucellar cap persists for a long time over the micropylar end of the embryo sac. Schwencke (1931) has pointed out that what Kanda considers to be nucellar tissue is really a part of the integument. The present work on *Lippia nodiflora* and *Duranta plumieri* supports the fact that the nucellus is absorbed at an early stage.

In most of the members of the Verbenaceae, worked out so far, the development

←

FIGS. 55-58 — Development of seed coat (*end*, endosperm; *int*, integument). Figs. 55, 56. Differentiation of testa. × 363. Figs. 57, 58. Meristematic activity of peripheral layers of endosperm; the inner part of the integument is consumed. × 564·5.



FIGS. 62-64 — Seed and fruit (*ch*, chalazal haustorium; *cv*, chalazal vascular trace; *emb*, embryo; *end*, endosperm; *f*, funiculus; *int*, integument; *mh*, micropylar haustorium; pc_1, outer region of pericarp with thin-walled cells; pc_2, inner region of pericarp with thick-walled cells; *ps*, persistent sepal; *st*, style). Fig. 62. L.S. young fruit × 52. Fig. 63. L.S. mature seed. × 52. Fig. 64. Part of Fig. 63 enlarged to show two persistent layers of endosperm lying next to the seed coat. × 520.

of the embryo sac is of the Polygonum type. In *Avicennia officinalis* Karsten (1891) reported Allium type of development but this requires confirmation.

Patermann (1935) states that a characteristic feature of the Verbenaceae is the quick degeneration of antipodal cells which begin to be absorbed before fertilization. Junell (1934) noted, however, that in some members of this family the antipodals divide and increase in number. In *Clerodendron phlomidis* a mass of 20 antipodal cells is reported (Misra, 1939). In *Lantana indica*, according to Thathachar (1940), the nuclei of the antipodal cells undergo repeated mitotic divisions and ultimately each cell becomes 3- to 6-nucleate. Later, the nuclei fuse and the cells themselves elongate and function as haustoria. In *Lippia nodiflora* and *Duranta plumieri* the antipodal cells persist for some time after fertilization but do not show any divisions.

The first division of the primary endosperm nucleus is followed by a transverse wall resulting in the formation of an upper primary micropylar and a lower primary chalazal chamber. Schwencke (1931) believes that the development of the endosperm may fall under two types, depending on the plane of division of the primary micropylar chamber which may be longitudinal or transverse. In fact, three main types of endosperm development have been distinguished in the Tubiflorales[1]. In the first, exemplified in *Scutellaria*, there is always a longitudinal wall in both chambers, micropylar and chalazal. In the second, *Brunella* type, the micropylar chamber divides by a longitudinal wall. In the third, *Stachys* type, the first wall in the micropylar chamber is transverse. *Verbena officinalis* (Souèges, 1935) and *Lippia nodiflora* (Pal, 1951; and present work) seem to show considerable variation, for here the first wall in the micropylar chamber may be transverse or longitudinal.

Kanda's (1920) report of a Helobial endosperm in *Verbena angustifolia* has

1. For detailed information on the endosperm haustoria of the Sympetalae, reference may be made to the recent publication of Crété (1951).

already been contradicted by Schnarf (1925) and Schwencke (1931). In fact, such an occurrence would be most unlikely since the Cellular type is of universal occurrence in both Labiatae and Boraginaceae.

With regard to the development of the endosperm haustoria in the Verbenaceae enough information is not available to permit much generalization. The chalazal haustorium is 2- or 4-nucleate and the primary chalazal chamber directly functions as a haustorium. Only in *Callicarpa macrophylla* (Junell, 1934) this haustorium consists of 4 longitudinally arranged cells. Cell divisions also occur in *Bouchea incrassata* (Junell, 1934).

The best developed micropylar haustoria occur in *Stachytarpheta* and *Bouchea* (Junell, 1934). In *S. dichotoma* the haustorium is formed of four large parallel cells and the same is true of *S. indica* (Thathachar, 1940). In *S. angustifolia* they divide to produce a larger number (8-12) of cells. In *Bouchea* it is 8-celled. In *Clerodendron fallax* (Junell, 1934) it consists of two unusually large cells each of which sends out a long branch penetrating the micropyle; and in *Verbena officinalis* and *Lantana camara* (Crété, 1942), of two cells which divide transversely to form four. In *Lippia* (Junell, 1934; present investigation) the haustorium is small and poorly developed.

Occasionally the development of the haustorium may vary in the same species. To quote an example, in *Stachytarpheta indica* Patermann (1935) described a binucleate micropylar haustorium whereas, according to Thathachar (1940), it is 4-celled.

Further work is necessary on the behaviour of the antipodal cells and the origin and differentiation of the endosperm haustoria.

Summary

The flowers are sessile and arranged on an oblong, compact spike. The floral parts arise in the sequence — sepals, stamens, petals and carpels.

The archesporium differentiates in the anther at the two-lobed stage. The anther wall comprises the epidermis, fibrous endothecium, a single middle layer and multinucleate glandular tapetum.

The reduction divisions are simultaneous and quadri-partition occurs by furrowing. Tetrahedral, decussate as well as isobilateral tetrads are formed. The mature pollen grains are two-celled.

The ovules are unitegmic, tenuinucellate and anatropous. The nucellus is single-layered and ephemeral. An integumentary tapetum differentiates around the embryo sac.

The hypodermal archesporial cell functions directly as the megaspore mother cell. The latter gives rise to a linear tetrad of megaspores. The chalazal megaspore functions and the development of the embryo sac is of the Polygonum type. The synergids degenerate soon after fertilization but the antipodal cells persist for some time.

The primary endosperm nucleus divides by a transverse wall giving rise to the primary micropylar chamber and the primary chalazal chamber. The latter functions directly as the chalazal haustorium which becomes two-nucleate. There are two to three transverse divisions in the micropylar chamber followed by a longitudinal division. The uppermost tier differentiates into the micropylar haustorium. This functions for a short time only, after which the cells below it and then those next below function similarly and behave as haustoria. In later stages, the peripheral layers of the endosperm proper show cambium-like divisions and become more prominent than the inner cells. Only the two outermost layers of the endosperm persist in the mature seed.

The first division of the zygote is transverse. The terminal cell divides longitudinally whereas the basal cell divides transversely. The development corresponds to the Crucifer type. The penultimate cell of the proembryo functions as the hypophysis initial. From its derivatives, differentiate the initials of the root cortex, root cap and the root epidermis.

The integument comprises six to seven layers of parenchymatous cells. The outer 3-4 layers show well developed reticulate thickenings while the cells of the inner 3-4 layers have cutinized walls. Only the

outer one or two layers of the integument persist in a mature seed.

The pericarp becomes differentiated into two regions — the outer of pitted cells and the inner of sclereids.

It gives me great pleasure to express my gratitude to Prof. P. Maheshwari and Dr. B. M. Johri for kindly suggesting this problem and guiding me throughout the course of this work.

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THE EMBRYOLOGY OF *TAMARIX* LINN.

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The family Tamaricaceae comprises 4 genera — *Tamarix, Myricaria, Reaumuria* and *Hololachna*. *Myricaria germanica* and several species of *Tamarix* have so far been investigated, but practically nothing is known about the other 2 genera.

The embryo sac is tetrasporic. Frisendahl's (1912) and Mauritzon's (1936) report of Adoxa type of embryo sac in *M. germanica* has been contradicted by Zabban (1936). According to him the embryo sac is of the Fritillaria type as has also been confirmed by Battaglia (1943). Mauritzon (1936) observed the Adoxa type of development in 6 species of *Tamarix* — *T. aestivalis, T. africana, T. gallica, T. odessana, T. pentandra* and *T. tetrandra*. He ignored the possibility of other types of embryo sacs as is clear from his statement: " Ich habe nicht versucht zu beobachten, ob wie bei *Myricaria* eine Abweichung von dem typischen Lilium-

Typus vorkommt ", and " Nicht selten findet man in Embryosack Kerne, deren Anzahl sowie der übrige Bau des Embryosackes es unmöglich machen, zu einer sicheren Deutung des Präparates zu gelagen ". What Mauritzon refers to as Lilium type now means Adoxa type since the genus *Lilium* comes under the Fritillaria type.

Several other species of *Tamarix* have also been worked out and the Fritillaria type occurs in *T. dioica* (Joshi & Kajale, 1936), *T. gallica* (Battaglia, 1941), *T. ericoides* (Sharma, 1939, 1940), *T. chinensis* (Puri, 1939) and *T. africana* (Battaglia, 1941). Battaglia further discovered that some of the embryo sacs in *T. gallica* and *T. africana* were of the Drusa, Adoxa and Chrysanthemum cinerariaefolium types. Thus the female gametophyte of *Tamarix* shows a good deal of variation and in view of these conflicting reports, Maheshwari (1937, 1946a, 1946b) suggested a re-investigation of this genus.

The development of endosperm and embryo has not been fully studied in any species of *Tamarix*, and this led us to take up the present work about three years ago.

The gametophytes of *T. pentandra* Pall. and *T. troupii* Hole, early embryogeny of *T. troupii* and the seed development of *T. ericoides* Rottl. have been studied.

Material and Methods

Prof. P. Maheshwari collected material of *Tamarix pentandra* from the Botanical Garden, Copenhagen, in July 1950, while he was in Europe to attend the 7th International Botanical Congress. *T. ericoides* was kindly sent by Mr. B. Tiagi (Ajmer) and *T. troupii*[1] was fixed locally from Timarpur (Delhi) during August-September 1951.

Formalin-acetic-alcohol was used for fixation and the material was subsequently stored in 70 per cent ethyl alcohol. Sections of *T. ericoides* were cut at 7-12 microns, those of *T. troupii* at 5-10 microns and of *T. pentandra* at 8 microns. Staining with safranin-fast green as well as iron-haematoxylin gave good results.

1. We are grateful to Sri M. B. Raizada of the Forest Research Institute, Dehra Dun, for confirming the identification of our specimens.

The Flower

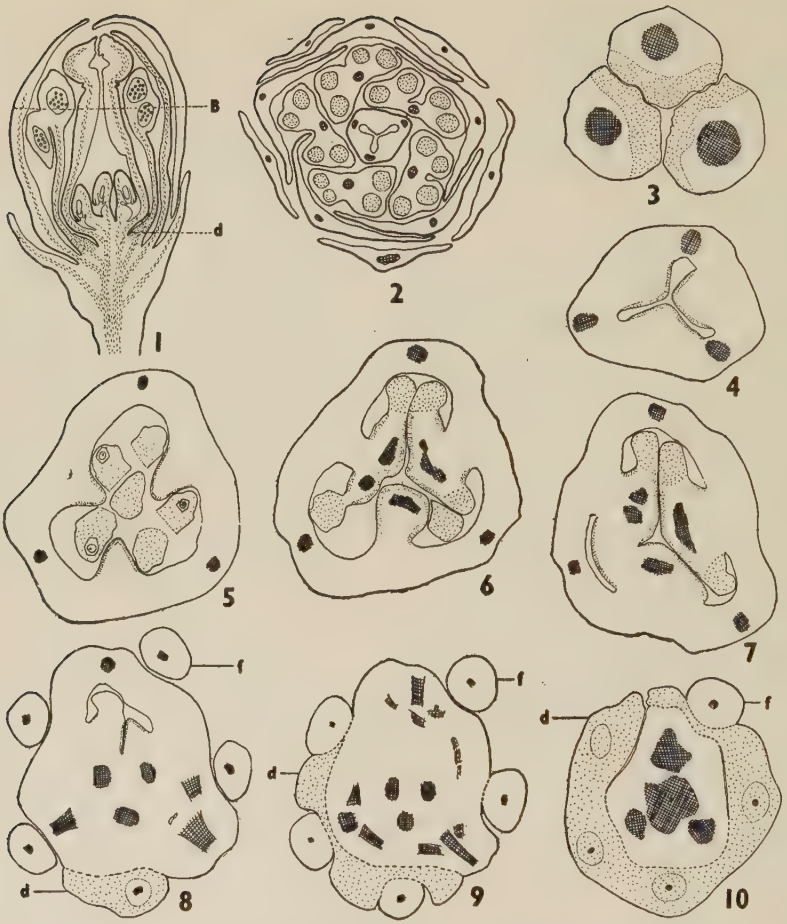
The floral structure of *T. pentandra* and *T. troupii* is essentially similar. Longitudinal and cross-sections of a flower bud and transverse sections of the gynaecium of *T. pentandra* are represented in Figs. 1-10.

The polypetalous, pentamerous, hypogynous flowers have a unilocular, superior ovary with several ovules on each of the three basal placentae (Figs. 1, 2, 5-7). The long hollow style terminates in a 3-lobed expanded stigma and has a triradiate canal lined with glandular epithelium (Figs. 1-4). At the base of the ovary is present a cup-shaped, lobed glandular disc. It is fused with the staminal filaments which do not all separate at the same time (Figs. 1, 8-10).

Microsporogenesis and Male Gametophyte

The wall of the anther comprises 5 layers — the epidermis, endothecium, 2 middle layers and the secretory tapetum (Fig. 11). The middle layers show signs of flattening even at the mother-cell stage. The inner degenerates quite early but the outer persists until 2-celled pollen grains have been formed (Fig. 13). At maturity, due to disorganization of the separating wall the 2 adjacent pollen sacs become confluent (Figs. 12, 14). The epidermis remains quite prominent and its outer wall acquires a thick cuticle. The endothecium develops the usual fibrous thickenings and an occasional cell undergoes a periclinal division (Fig. 13). In the region of dehiscence 2 to 3 rows of cells of the epidermis and the endothecium remain narrow and the latter are devoid of any fibrous thickenings (Fig. 15, *d*).

The tapetal cells are at first uninucleate but due to mitotic divisions they become 2- to 3-nucleate (Fig. 11). The nuclei may fuse irregularly and divide again resulting in one or more large, amoeboid, polyploid nuclei. The tapetum disorganizes during the formation and maturation of the 2-celled pollen grains (Figs. 13, 15).



FIGS. 1-10 — *Tamarix pentandra* (*d*, disc; *f*, filament). Fig. 1. L.S. flower bud (diagrammatic). × 24. Fig. 2. T.S. younger bud approximately at level B marked in Fig. 1. × 100. Figs. 3-10. Serial cross-sections of gynaecium from apex to base. × 100.

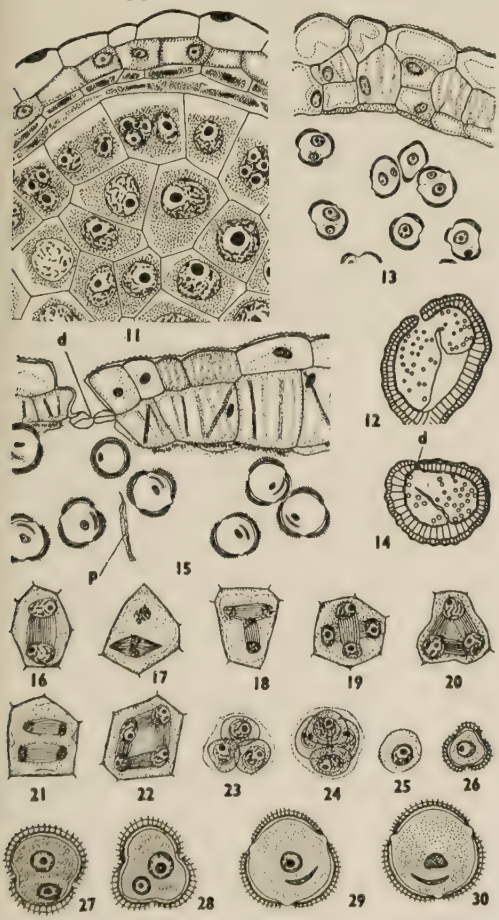
Just prior to meiosis the protoplasts of the mother cells contract and a mucilaginous special wall is secreted between the protoplast and the original wall. The reduction divisions are simultaneous and the spindles may lie parallel or at right angles to each other (Figs. 16-21). Secondary spindle fibres appear after Meiosis II (Fig. 22). The tetrads may be isobilateral, tetrahedral (Fig. 23) or decussate (Fig. 24). Cytokinesis takes place by centripetal furrows followed by wedges of the mucilaginous wall which meet in the centre and bring about quadripartition. Finally the mucilage is consumed and the microspores are liberated.

The wall of the young microspore soon differentiates into an intine and an exine (Figs. 25, 26). At the 2-celled stage the generative and the vegetative cells are separated by an ephemeral membrane (Fig. 27) which later dissolves (Figs. 28, 29). In Fig. 30 the tube nucleus has degenerated and lies near the generative cell. The mature pollen is tricolpate and subpyrolate[2] (the polar axis and the equatorial diameter being more or less equal). The exine is baculate (shows rod-like thickenings) and appears reticulate in surface view. Erdtman (1952),

2. Terminology according to Erdtman (1952).

who has described the pollen grains of *Myricaria germanica, Reaumuria hirtella, Tamarix gallica* and *T. tetrandra,* states that they are somewhat similar to those of the Frankeniaceae as well as to some of the pollen types in the Rhoeadales.

Sharma's (1939) statement that in *T. ericoides* the anther tapetum is " formed by the outermost layer of the sporogenous tissue " appears to be erroneous. In *T.*



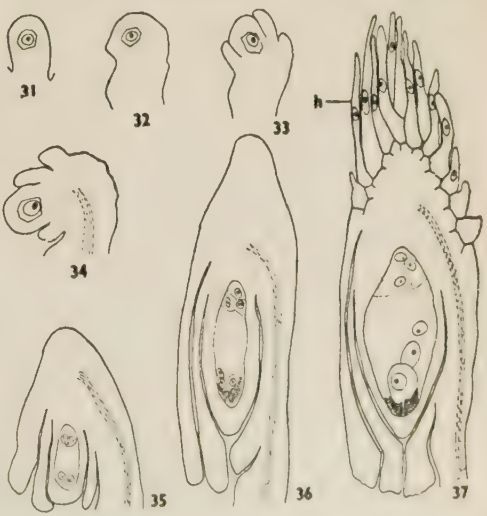
FIGS. 11-30 — Figs. 11-13, 16-28 of *T. pentandra* and Figs. 14, 15, 29, 30 of *T. troupii* (*d*, region of dehiscence; *p*, remains of partition wall between pollen sacs). Fig. 11. Part of anther wall with microspore mother cells. × 588. Fig. 12. Mature anther lobe. × 22. Fig. 13. Part of same, enlarged. × 383. Figs. 14, 15. Same as Figs. 12 and 13 but of *T. troupii*. Figs. 16-22. Mother cells, Meiosis I and II. × 588. Figs. 23, 24. Tetrahedral and decussate tetrads. × 588. Figs. 25, 26. Uninucleate microspores. × 588. Figs. 27-30. Two-celled pollen grains. × 766.

pentandra and *T. troupii* it is derived as usual from the parietal tissue. Battaglia (1941) reports polyspory in *T. gallica* but we did not come across any such case either in *T. troupii* or in *T. pentandra.*

Ovule

The ovule is anatropous. It arises as a small erect protuberance but simultaneously with the differentiation of the megaspore mother cell and the integuments it begins to curve and finally becomes anatropous (Figs. 31-35). Figs. 36, 37 show further development of the integuments. The inner arises first, grows faster than the outer and forms the micropyle (Figs. 33-37). Both the integuments remain separate from each other and from the nucellus. They are 2-layered except at the micropylar end where the inner integument becomes 3 to 4 cells thick. At the chalazal end the epidermal cells elongate and give rise to a hairy growth (Fig. 37, *h*). Joshi & Kajale (1936) report the same condition in *T. dioica.*

The nucellus is thin and at the megaspore mother-cell stage it consists of only two layers of cells including the epidermis (Figs. 40, 50). With the enlargement of the embryo sac the inner layer is crushed soon after fertilization. This is also the



FIGS. 31-37 — *T. pentandra* (*h*, hairs). Development and orientation of ovule from archesporial cell to mature embryo sac stage. × 227.

case in *T. tetrandra* (Mauritzon, 1936). The funicular strand is quite prominent and terminates at the chalaza (Figs. 35-37).

Megasporogenesis and Female Gametophyte

The hypodermal archesporial cell (Figs. 38, 49) (sometimes two cells may be present) cuts off a wall cell which divides anticlinally (Figs. 39, 40, 50). A wall cell is also formed in other species of *Tamarix*[3] (see Schnarf, 1931; Joshi & Kajale, 1936; Mauritzon, 1936; Sharma, 1939; Puri, 1939; Pàroli, 1939; Battaglia, 1941).

Frisendahl (1912) reported the absence of a wall cell in *Myricaria germanica* but Mauritzon (1936) has shown that it is formed here also and we are able to confirm it.

The megaspore mother cell enlarges and undergoes the usual meiotic divisions giving rise to 4 megaspore nuclei (Figs. 41, 51). Wall formation does not occur either after the first or the second division so that the daughter nuclei lie free within the elongated mother cell. Since all the megaspore nuclei take part in the formation of the embryo sac, the development is tetrasporic. The nuclei take up varying positions and their arrangement determines subsequent development. The following positions were observed:

1. Three nuclei may lie at the chalazal and 1 at the micropylar end (Figs. 42, 52). This 1+3 arrangement may lead to the Fritillaria or the Drusa type of development.

2. One or more vacuoles may separate the 2 chalazal from the 2 micropylar nuclei (Figs. 45, 56). Such a 2+2 arrangement would lead to the Adoxa type.

3. Two large vacuoles may appear at the 2 poles pushing 2 nuclei to the centre and leaving one each at the micropylar and the chalazal ends. This 1+2+1 arrangement gives rise to the Chrysanthemum cinerariaefolium type.

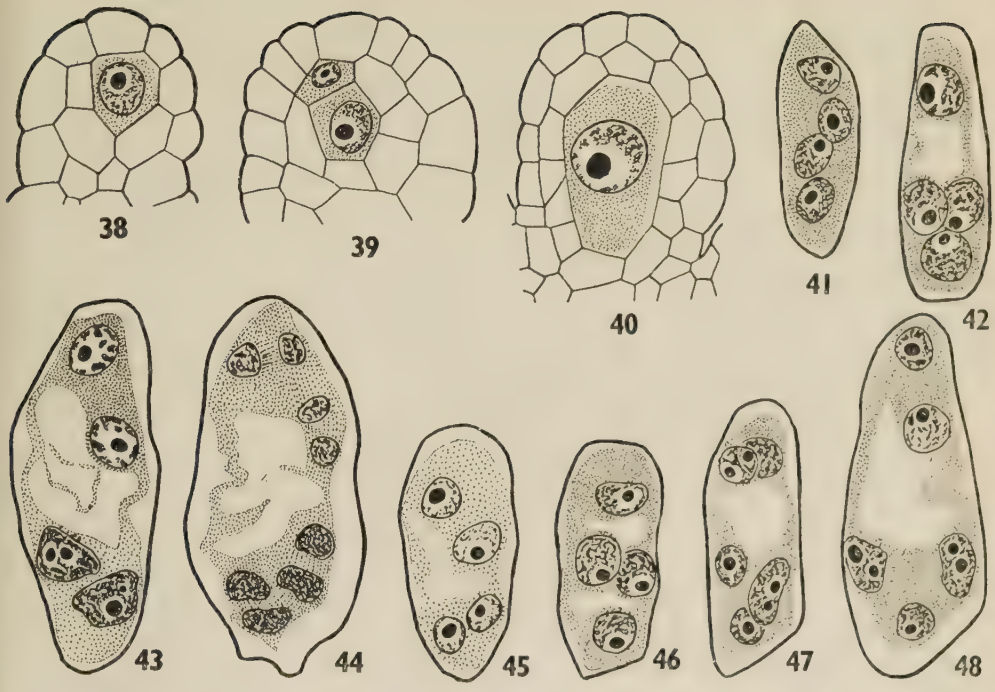
The development according to each of the 4 types named above may be taken up one by one:

Fritillaria Type — The 1+3 arrangement of the megaspore nuclei represents the primary 4-nucleate embryo sac in which all the nuclei are of the same size (Figs. 42, 52). The 3 chalazal nuclei lie close together and may even overlap. Figs. 43 and 53 show secondary 4-nucleate embryo sacs with 2 smaller (haploid) micropylar nuclei and 2 larger (presumably triploid) chalazal nuclei. Although the fusion of the 3 chalazal spindles was not observed, comparison with other species of *Tamarix* and the larger size of the 2 lower nuclei which may often show 3 nucleoli each (Fig. 53) leave little doubt that this interpretation is correct. Another division results in the formation of an 8-nucleate embryo sac with 4 haploid micropylar and 4 triploid chalazal nuclei (Fig. 44). The former organize into the egg apparatus and the upper polar nucleus while the chalazal group produces the lower polar nucleus and the 3 antipodal cells. Fig. 54 probably represents such an embryo sac where each of the antipodal cells has undergone a nuclear division.

Drusa Type — One further division after the primary 4-nucleate (1+3) stage results in 2 nuclei at the micropylar end and 6 at the chalazal end — all haploid. These 8 nuclei undergo another division to form a 16-nucleate embryo sac (Fig. 55). The egg apparatus is organized as usual, 2 nuclei function as polars, and 11 as antipodals. Occasionally, some of the chalazal nuclei of the 8-nucleate embryo sac fail to divide so that the final number at this end may be 12, 10, 8 or still fewer. This type of development was observed in *T. pentandra* but not in *T. troupii*.

Adoxa Type — The Adoxa type of embryo sac has been observed in *T. troupii* as well as *T. pentandra*. Here all the 4 nuclei of the primary 4-nucleate (2+2) stage (Figs. 45, 56) divide once to form 8 nuclei, 4 at each pole of the embryo sac. The micropylar quartet gives rise to the egg apparatus and the upper polar nucleus while the chalazal quartet forms the lower polar nucleus and the three antipodal cells. The embryo sac

3. Joshi and Kajale (1936) wrongly state that Dahlgren (1927) observed a parietal cell in *Tamarix tetrandra*. In fact Dahlgren mentions nothing about *Tamarix* but has only pointed out, on the basis of a personal communication from Dr. A. Frisendahl, that a wall cell is not formed in *Myricaria*.

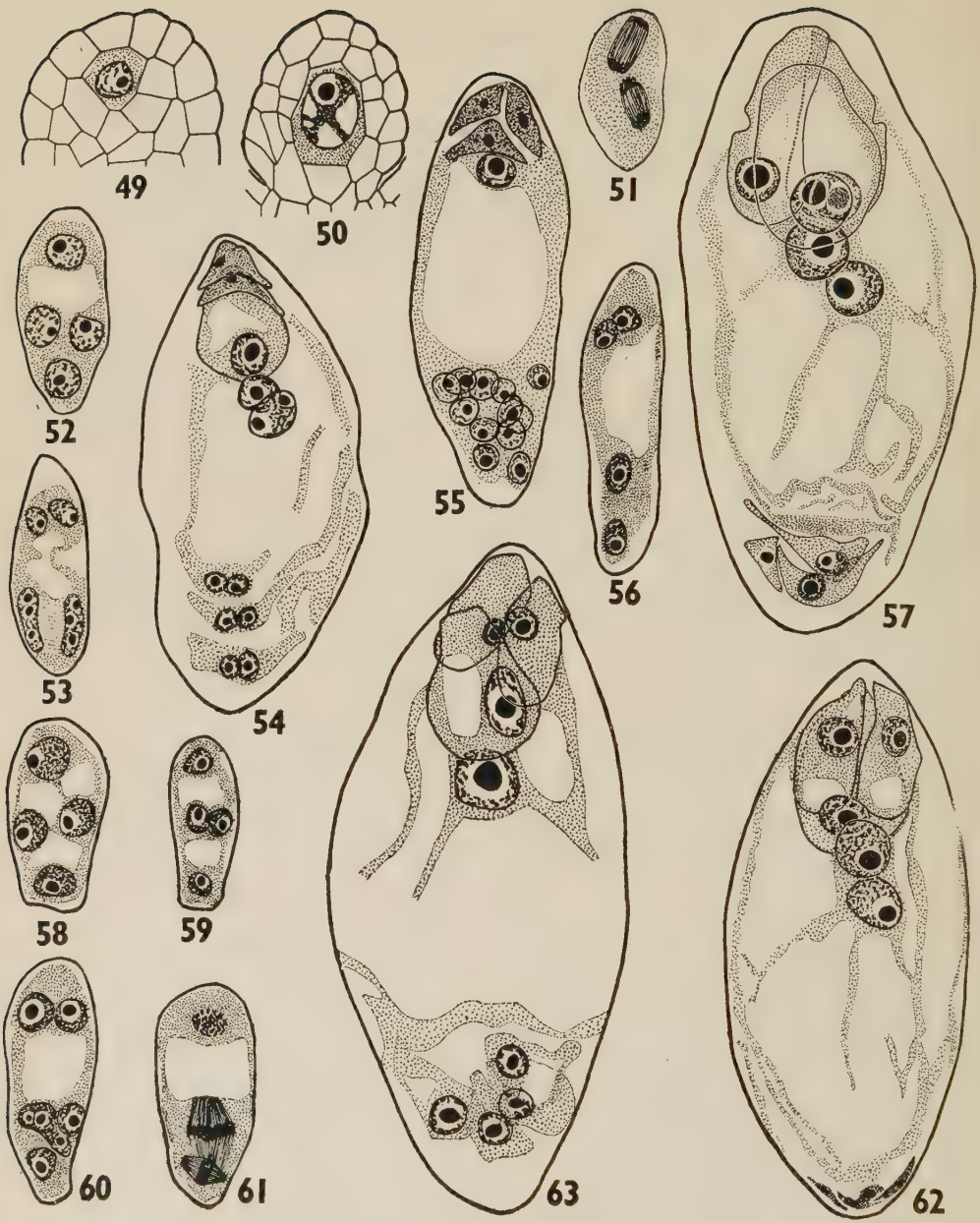


FIGS. 38-48 — *T. troupii*. Fig. 38. Young nucellus with hypodermal archesporial cell. Fig. 39. Parietal cell and megaspore mother cell. Fig. 40. Megaspore mother cell in prophase of Meiosis I. Fig. 41. Primary 4-nucleate embryo sac. Fig. 42. Same, showing 1+3 arrangement. Fig. 43. Secondary 4-nucleate stage. Fig. 44. Eight-nucleate embryo sac (Fritillaria type). Fig. 45. Primary 4-nucleate stage with 2+2 arrangement of megaspore nuclei. Fig. 46. Same, showing 1+2+1 arrangement. Fig. 47. Embryo sac with 2 haploid nuclei at micropylar end, and 1 diploid and 2 haploid nuclei at chalazal end. Fig. 48. Same, showing 2 haploid nuclei at micropylar end, and 1 haploid and 2 diploid nuclei at chalazal end. × 996.

shown in Fig. 57 may have been derived by this mehtod.

CHRYSANTHEMUM CINERARIAEFOLIUM TYPE — Sometimes the megaspore nuclei show a 1+2+1 arrangement (Figs. 46, 58). The 2 central nuclei come very close together (Fig. 59) and may fuse as in *Chrysanthemum cinerariaefolium* (Martinoli, 1939). Figs. 47 and 60 show embryo sacs with a large chalazal nucleus and 4 smaller nuclei—2 at each pole. Fig. 48 represents another embryo sac with 2 micropylar and 3 chalazal nuclei. Of the latter, 2 are binucleolate and larger than the third. The embryo sac in Fig. 61 shows one smaller spindle (top view) at the micropylar end and 2 spindles (side view), of which one is larger than the other, at the chalazal end.

These figures indicate that the 2 central nuclei of the primary 4-nucleate (1+2+1) stage have fused to form a diploid nucleus which has moved to the chalazal end. Thus the embryo sac shows a haploid nucleus at the micropylar end and 1 diploid and 1 haploid nucleus at the chalazal end. Two further divisions would produce a 12-nucleate embryo sac with 4 haploid micropylar and 4 diploid and 4 haploid chalazal nuclei. Such an embryo sac would have a haploid egg apparatus and upper polar nucleus while the lower polar nucleus would be diploid. Out of the 7 antipodals 3 would be diploid and 4 haploid. Similar embryo sacs were discovered by Battaglia (1941) in *T. gallica* and *T. africana*.



FIGS. 49-63 — *T. pentandra.* Fig. 49. Hypodermal archesporial cell. Fig. 50. Megaspore mother cell. Fig. 51. Same, Meiosis II. Fig. 52. Megaspore nuclei, 1+3 arrangement. Fig. 53. Secondary 4-nucleate stage. Fig. 54. Fritillaria type of embryo sac with binucleate antipodal cells. Fig. 55. Drusa type. Fig. 56. Four-nucleate stage showing 2+2 arrangement of megaspore nuclei. Fig. 57. Adoxa type of embryo sac. Figs. 58, 59. A 1+2+1 arrangement of megaspore nuclei. Fig. 60. Embryo sac with 2 haploid nuclei at micropylar end, and 1 diploid and 2 haploid nuclei at chalazal end. Fig. 61. Same, showing haploid spindle at micropylar end, and 1 haploid and 1 diploid spindle at chalazal end. Fig. 62. Mature embryo sac with degenerated antipodal cells. Fig. 63. Same, with 4 healthy antipodal cells. × 717.

The percentage of different types of embryo sacs in *T. troupii* and *T. pentandra* is approximately as follows:

	Fritillaria type	Drusa type	Adoxa type	Chrysanthemum cinerariaefolium type
T. troupii	73%	Nil	14%	13%
T. pentandra	38%	8%	48%	6%

Mature Embryo Sac — There is the usual 3-celled egg apparatus, 2-5 polar nuclei and 3-11 antipodals. The egg protrudes below the synergids and shows a prominent vacuole (Figs. 54, 57, 63). The synergids are broad and hooked, and often egg-like (Fig. 57). The latter condition has also been observed in *Myricaria germanica* (Frisendahl, 1912), *Tamarix dioica* (Joshi & Kajale, 1936) and *T. ericoides* (Sharma, 1939).

The upper polar nucleus is almost always haploid but the lower may be haploid (Adoxa and Drusa types), diploid (Chrysanthemum cinerariaefolium type) or triploid (Fritillaria type).

The antipodals usually remain uninucleate (Figs. 57, 62) but sometimes they become bi-nucleate (Fig. 54), or they may enlarge and show vacuolated cytoplasm (Fig. 63). In *T. tetrandra* (Mauritzon, 1936) also the antipodal cells elongate and become vacuolated after fertilization.

Occasionally the synergids (Figs. 54, 55) or the antipodals may collapse before fertilization (Fig. 62) and rarely the egg may also be involved (Fig. 55). Some of the embryo sacs show arrested growth and degenerate completely. In the latter case the contents take a dark stain and the structure is difficult to ascertain.

Pollination and Fertilization

Figs. 64 and 65 show germinating pollen grains on the stigma. As the pollen grain germinates, the tube nucleus takes the lead (Fig. 66). The male cells are spindle-shaped and show a distinct cytoplasmic sheath (Figs. 67, 68).

The pollen tube passes through the micropyle, enters the embryo sac and demolishes one of the synergids. Occasionally 2 pollen tubes enter (Figs. 69, 70) and crush both the synergids or one of them may be fertilized so as to result in the formation of an additional embryo (Fig. 69). The discharged pollen tube shows ' x '-bodies which take a bright-red stain with safranin-fast green (Figs. 69, 70).

At the time of fertilization the cells of the nucellar epidermis, particularly at the apex, are richly cytoplasmic and glandular. Normally the nucellus is completely covered by the inner integument but rarely it may project beyond the micropyle due to failure of proper development of the integuments (Fig. 71).

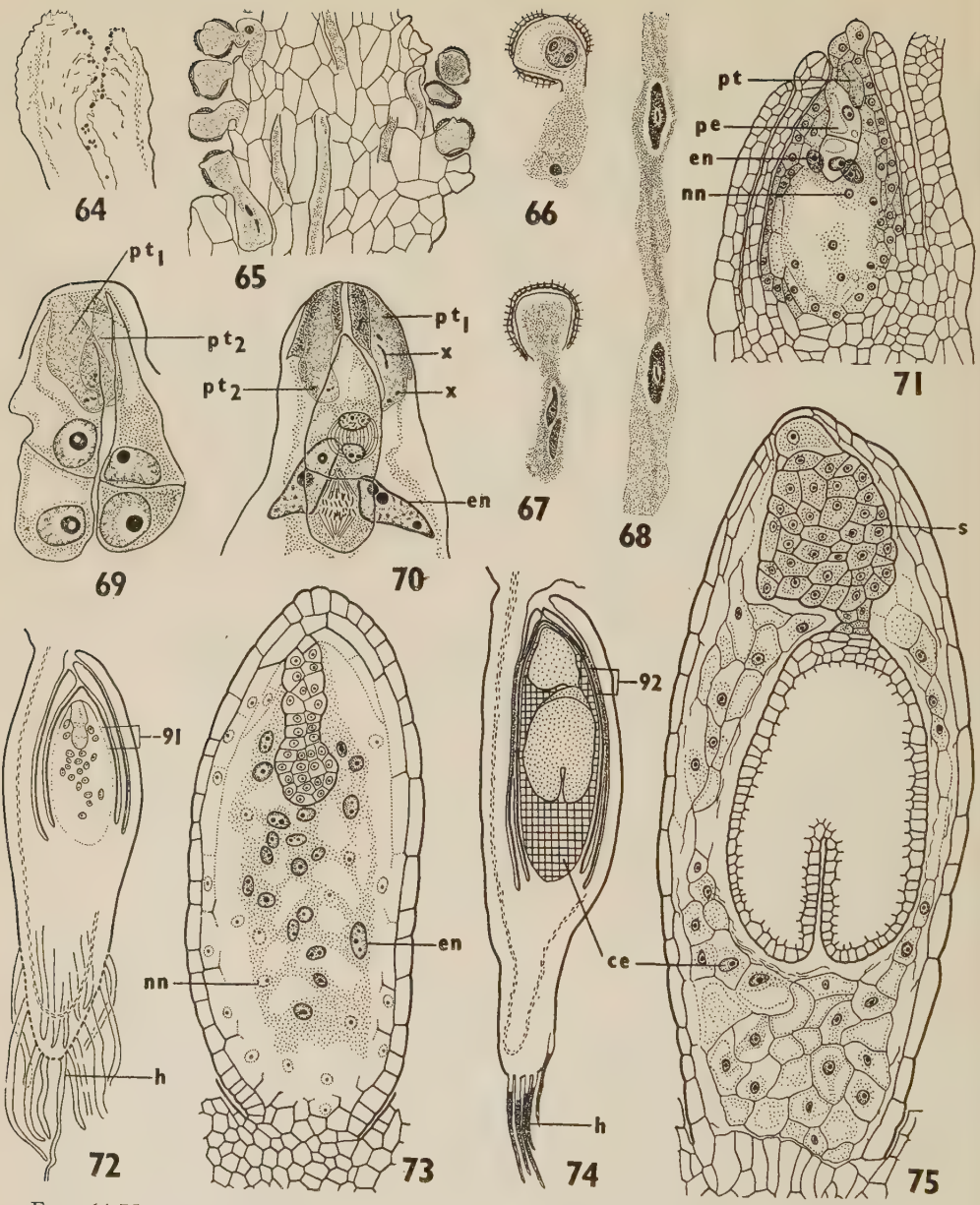
Endosperm[4]

In all the species under investigation — *T. pentandra*, *T. troupii* and *T. ericoides* — the endosperm is Nuclear. It has been more fully studied in *T. ericoides* but the earlier development is more or less identical in all the three species.

The primary endosperm nucleus usually divides only slightly before the zygote. Fig. 71 shows a 2-celled proembryo and 2 endosperm nuclei. In Fig. 70 both the cells of the proembryo are dividing but the number of endosperm nuclei is the same. The latter divide repeatedly giving rise to many free nuclei (Figs. 72, 73), 40 having been counted in *T. ericoides*. Cell formation is initiated in *T. troupii* while the proembryo is still globular but in *T. ericoides* it occurs at the heart-shaped stage[5]. Finally, as the cotyledons differentiate, the entire embryo sac becomes filled with cellular endosperm (Figs. 74, 75). This is consumed during matura-

4. A brief account of the development of the endosperm in *Tamarix* has already appeared elsewhere (Johri, 1954).

5. Due to inadequacy of material, endosperm development could not be fully studied in *T. pentandra*.

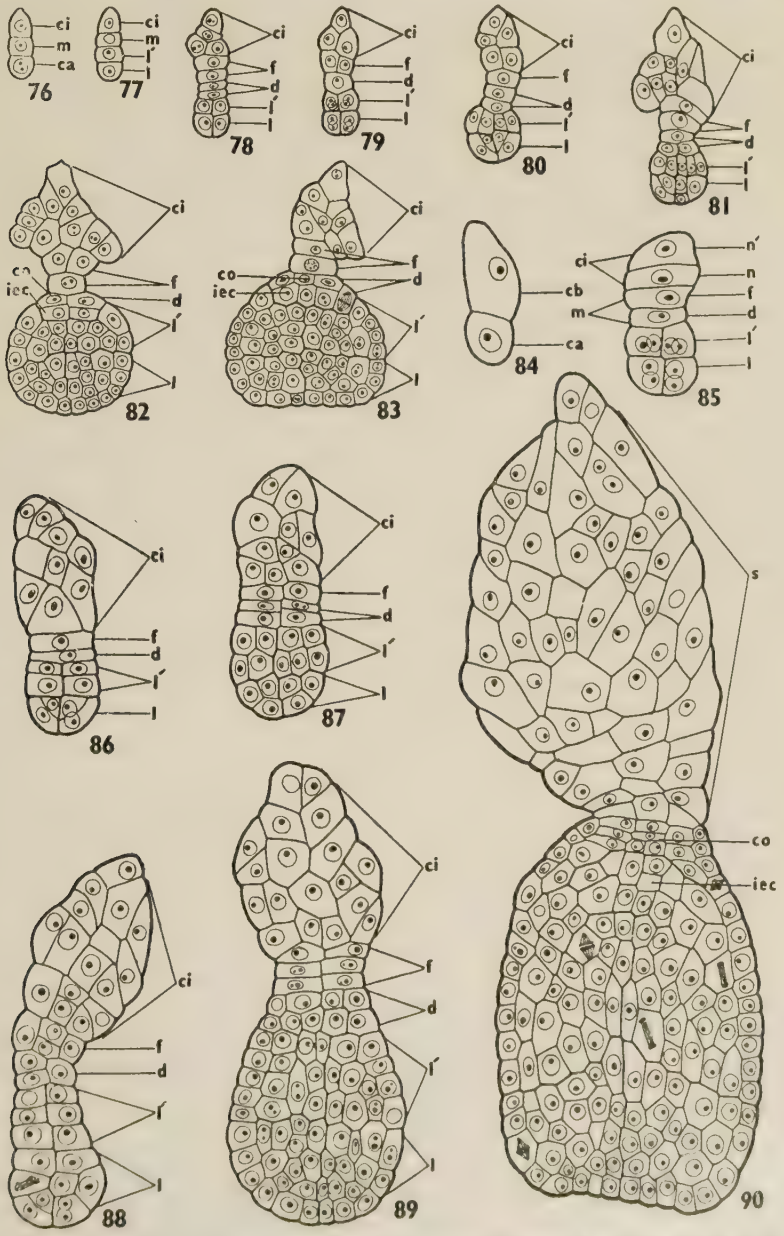


FIGS. 64-75 — *T. ericoides* (*ce*, cellular endosperm; *en*, endosperm nuclei; *h*, hair; *nn*, nucellar nuclei; *pe*, proembryo; *pt*, pollen tube; *s*, suspensor; *x*, x-bodies). Fig. 64. Stigma studded with germinating pollen grains. × 28. Fig. 65. Part of same, enlarged. × 466. Figs. 66, 67. Germinating pollen grains (from stigma), the latter figure shows 2 ♂ cells in the pollen tube. × 466. Fig. 68. Part of pollen tube with ♂ cells (from stigma). × 466. Figs. 69, 70. Upper parts of embryo sacs showing 2 pollen tubes in each; in the former, one of the synergids has developed into a 2-celled proembryo. × 466. Fig. 71. L.S. upper part of ovule with nucellus projecting beyond the integuments. Cell walls of the inner layer have broken down and the nuclei have migrated into the embryo sac. × 289. Fig. 72. L.S. ovule at early globular stage of proembryo; epidermal cells at the chalazal end form a plume. × 39. Fig. 73. Nucellus with embryo sac, shown in Fig. 72, enlarged; nuclei of nucellar cells are mixed up with the endosperm nuclei. × 118. Fig. 74. L.S. ovule at a stage when the endosperm has become cellular; part of the inner integument has been crushed; walls of hairs at chalazal end have become thickened. × 39. Fig. 75. Nucellus and embryo sac, shown in Fig. 74, enlarged to show detailed structure of embryo and cellular endosperm. × 118.

tion of the seed which is thus exalbuminous (Figs. 95, 96).

Mauritzon's (1936), Sharma's (1939) and Puri's (1939) accounts of endosperm formation in *T. tetrandra*, *T. ericoides* and *T. chinensis* respectively need modification and would be considered in the ' Discussion '.

After fertilization the layer of nucellar cells in immediate contact with the embryo sac breaks down and their nuclei migrate into the embryo sac (Figs. 71,



Figs. 76-90 — Development of embryo. Figs. 76-83 of *T. troupii* and Figs. 84-90 of *T. ericoides*. For explanation see text. × 311.

73). They can be easily identified from the endosperm nuclei by their smaller size and often also by their uninucleolate condition. Gradually with the increase in the number of the endosperm nuclei, the nucellar nuclei degenerate until none are recognizable at the advanced globular stage of the proembryo. This feature has not been reported earlier in any species of *Tamarix* but occurs in some other plants (see Maheshwari, 1950).

Embryo

The zygote divides transversely forming the terminal cell *ca* and the basal cell *cb* (Fig. 84). Usually both the cells divide simultaneously (Fig. 70) but sometimes *cb* may divide earlier than *ca* (Fig. 76). The daughter cells of *ca* have been designated *l* and *l'* while those of *cb* as *m* and *ci* (Figs. 76, 77). The next two divisions in *l* and *l'* are by longitudinal walls, oriented at right angles to each other, resulting in the quadrant and octant stages (Figs. 78, 79, 85). The tiers *l* and *l'* divide periclinally cutting off the dermatogen (Fig. 80). In Fig. 86 the tier *l* has divided periclinally and *l'* has divided transversely.

Meanwhile, the cell *m* divides transversely giving rise to *d* and *f* which undergo further divisions, transverse or longitudinal (Figs. 78-80); while *ci* divides to from *n* and *n'* (Fig. 85). Later divisions are irregular and in *Tamarix troupii* the peripheral cells of the suspensor bulge out prominently (Figs. 78-83, 86-90).

The derivatives of *ci* and *f* produce a massive suspensor of more or less vesicular cells (Fig. 90, *s*). It is not so well developed in *T. troupii* (Figs. 78-83) as in *T. ericoides* (Figs. 86-90). The suspensor cells are richly cytoplasmic, contain prominent nuclei and store abundant starch in later stages. They have a haustorial function and draw upon the adjacent cells of the nucellus as well as the endosperm (Fig. 75). Their remains persist in the mature seed (Figs. 95, 97, *s*).

The cell *d* undergoes a transverse and then a longitudinal division; or the sequence of divisions may be in the reverse order, ultimately resulting in two tiers *co* and *iec* (Figs. 78, 81-83, 87-89). The former gives rise to the root cap and the latter to the initials of the central cylinder of root (Fig. 90).

After delimitation of the dermatogen, the tiers *l* and *l'* divide repeatedly so that at first a globular and then a heart-shaped embryonal mass is produced (Figs. 82, 83, 89, 90). Further growth results in the formation of the hypocotyl, cotyledons and the stem tip.

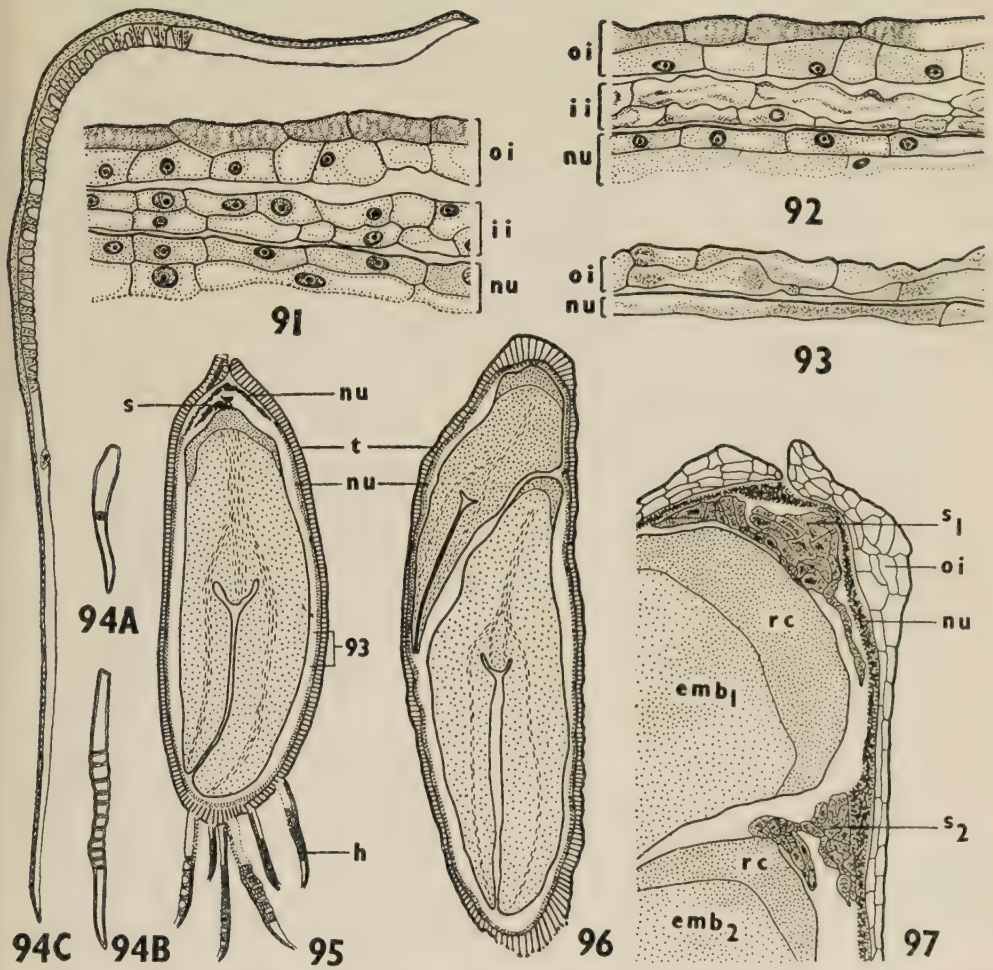
The sequence of development in *T. troupii* is similar to that in *Helianthemum guttatum* (Souèges, 1937), while *T. ericoides* seems to resemble *Linum catharticum* (Souèges, 1924). *H. guttatum* as well as *L. catharticum* belong to the same embryogenic group.

Polyembryony — Several cases of polyembryony were noticed (Figs. 69, 96). The presence of 2 pollen tubes is of common occurrence and the additional embryo develops from one of the synergids (Fig. 69). Sharma (1939) has also recorded polyembryony in the same species — *Tamarix ericoides*. From his Fig. 42 it appears that besides the zygote both the synergids also develop into embryos. Traub (1939) reports polyembryony in *Myricaria cauliflora* in which he observed 1-6 seedlings arising from one seed. No developmental studies were made, however.

Seed

During post-fertilization stages the integuments and nucellus undergo considerable changes and modifications. The tangential walls of the outer layer of the outer integument become thickened and pitted. After the appearance of the cotyledons the inner integument collapses, first in the middle and then at the two ends (Figs. 74, 92). Finally, in the mature seed only the outer integument survives and forms the testa (Figs. 93, 95-97).

The epidermal cells at the chalazal end of the ovule elongate to form uniseriate hairs (Figs. 72, 74). At first these are 1-celled (Fig. 94A) but as the cotyledons differentiate they undergo transverse divisions to form filaments of cells. The basal and the apical cells become considerably



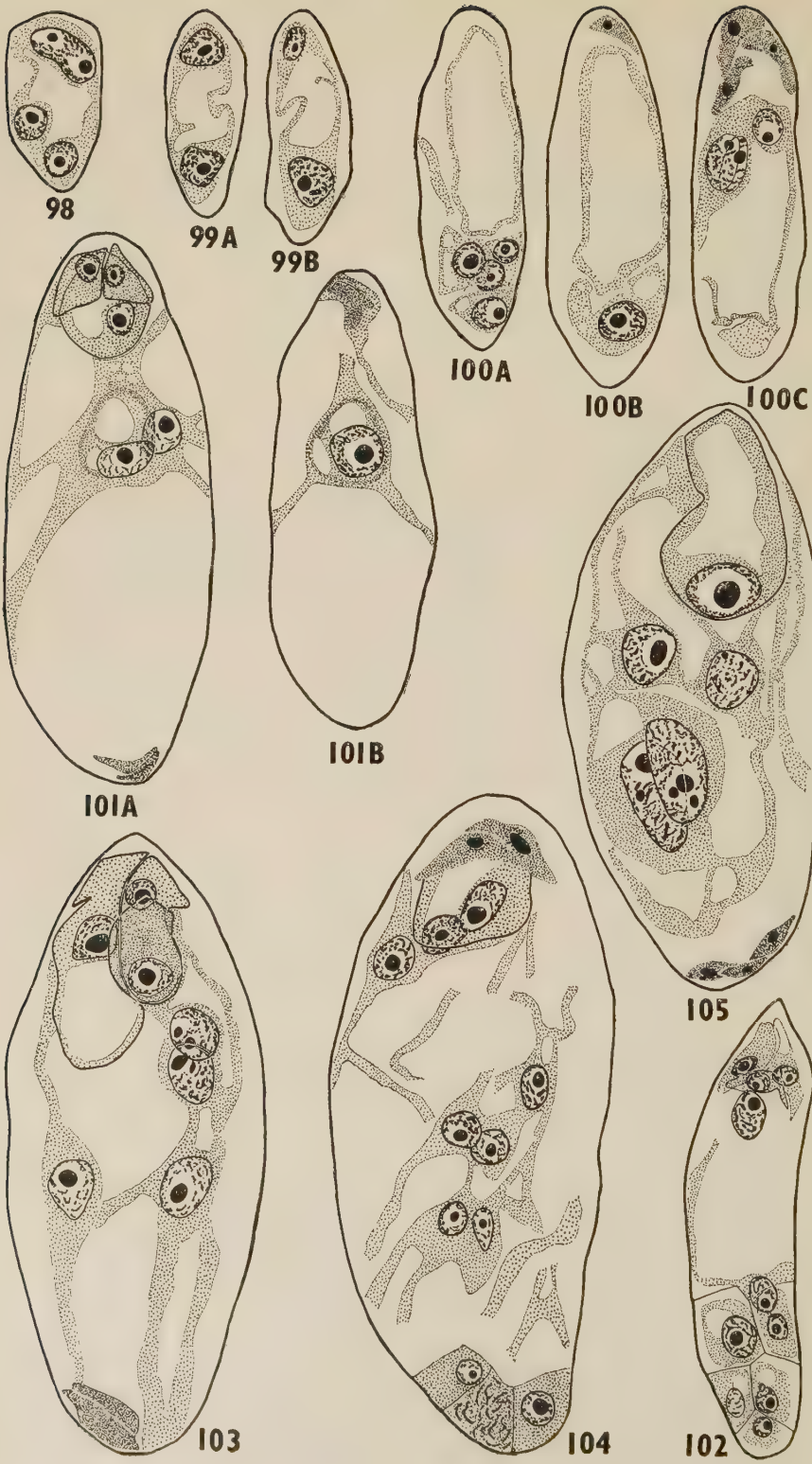
FIGS. 91-97 — *T. ericoides* (*emb*, *embryo*; *h*, hair; *ii*, inner integument; *nu*, nucellar remains (perisperm); *oi*, outer integument; *rc*, root cap; *s*, suspensor; *t*, testa). Figs. 91-93. Transformation of integuments and nucellus into testa and perisperm; position of Figs. 91, 92 and 93 marked in Figs. 72, 74 and 95 respectively. × 346. Figs. 94A, B, C. Development of hair. × 155. Fig. 95. L.S. normal seed. × 32. Fig. 96. Abnormal seed with 2 embryos. × 47. Fig. 97. Part of Fig. 96 enlarged (from a different successive section) to show persistent suspensors of both the embryos. × 346.

longer than the others and at maturity the hairs develop characteristic thickenings (Figs. 94B, 94C). One of the tangential walls and the radial walls become highly lignified whereas the other tangential wall remains thin (Fig. 94C).

It has already been pointed out that the inner layer of the nucellus breaks down by the time the proembryo is at the early globular stage (Figs. 73, 91, 92). The outer layer alone persists in the seed and forms the scanty perisperm (Figs. 93, 95-97). Sharma (1939) refers to a "loose thin-walled perisperm" but does not mention if it is derived from only one or both layers of the nucellus.

Abnormal Embryo Sacs

Several variations were observed in *Tamarix pentandra*.



Figs — 98-105.

Fig. 98 represents an embryo sac with 2 smaller uninucleolate chalazal nuclei and a large binucleolate micropylar nucleus. The latter has probably been formed as a result of fusion of the 2 upper nuclei of the primary 4-nucleate (2+2) stage. Such a condition would lead to the formation of a diploid egg apparatus.

Fig. 99A, B shows a 4-nucleate embryo sac. The 2 nuclei at the chalazal and 1 nucleus at the micropylar end are approximately twice the size of the fourth micropylar nucleus. Probably the 3 larger ones are diploid. The origin of the smallest nucleus is not clear.

Fig. 100A, B, C shows an 11-nucleate embryo sac with 4 degenerated cells at the upper end, 3 free nuclei in the centre and 4 cells at the chalazal end. Of the last, one is binucleate. This may be an embryo sac of the Drusa type in which some of the chalazal nuclei failed to divide and 2 other nuclei, besides the lower polar nucleus, had moved upwards.

Figs. 101A, B and 102 may be embryo sacs of the Adoxa type. In Fig. 101 it is presumed that one of the antipodal nuclei (much larger than the others) migrated upwards and came to lie along with the 2 polars. Two nuclei are enclosed in a cytoplasmic vesicle while the third is slightly displaced and is lying partly outside the vesicle. In Fig. 102 the lower polar nucleus is still at the chalazal end and 2 of the antipodal cells have divided.

Fig. 103 may represent another embryo sac of the Adoxa type or perhaps of the Chrysanthemum cinerariaefolium type. If the former, 2 of the antipodal nuclei moved up and the third antipodal divided once followed by a degeneration of both the daughter cells. However, judging from their size and appearance, the 2 overlapping nuclei lying just below the egg (presumably representing the two polars) as well as the 2 lower nuclei appear to be diploid. If so, these may have originated by two divisions of the central diploid nucleus formed after the primary 4-nucleate (1+2+1) stage. The micropylar nucleus must also have divided twice to give rise to the egg apparatus but the fourth (upper polar) nucleus is not traceable. The chalazal nucleus probably divided only once to form the two antipodal cells.

Fig. 104 may be a reduced 13-nucleate embryo sac of the Drusa type. Both the polars are lying adjacent to the egg apparatus, there are 3 antipodal cells (one of which is degenerating) and 5 free nuclei in the centre. The latter probably moved up from their position at the chalazal end.

More difficult to interpret is the embryo sac shown in Fig. 105. This is devoid of synergids but shows 4 free nuclei of which 2 are larger and perhaps diploid. The 3 antipodal cells have degenerated but are still clearly visible at the base of the embryo sac.

Another remote probability is that the embryo sacs represented in Figs. 101A, B and 103-105 may be post-fertilization stages and the free nuclei may be the endosperm nuclei. However, no trace of the pollen tube was found in any of these embryo sacs. The rarity of the abnormal embryo sacs makes any definite interpretation quite difficult.

Discussion

The variations in the origin and organization of the embryo sac and endosperm in *Myricaria* and *Tamarix* and the systematic position of the Tamaricaceae are discussed below.

FIGS. 98-105 — *T. pentandra*. Fig. 98. Embryo sac showing 1 large (diploid) micropylar and 2 smaller (haploid) chalazal nuclei. Fig. 99A, B. Four-nucleate embryo sac, one of the micropylar nuclei is of smaller size. Fig. 100A, B, C. Embryo sac with 4 degenerated micropylar cells, 3 central nuclei and 4 antipodal cells of which 1 is binucleate. Fig. 101A, B. Embryo sac with 3-celled egg apparatus, 3 nuclei in a central cytoplasmic vesicle, and two degenerated antipodals. Fig. 102. Egg apparatus, 5 antipodal cells and 2 polar nuclei. Fig. 103. Embryo sac with one synergid larger than the other, 4 central nuclei and 2 degenerated antipodals. Fig. 104. Same, showing degenerated synergids, healthy egg, 7 other nuclei and 3 antipodal cells. Fig. 105. Embryo sac with 4 nuclei of unequal size and 3 degenerated antipodals; synergids are absent. × 719.

Embryo Sac — Frisendahl's (1912) and Mauritzon's (1936) report of an Adoxa type of embryo sac in *M. germanica* is erroneous. The former observed the 1+3 arrangement of the megaspore nuclei as well as the secondary 4-nucleate stage. He explained these as abnormalities caused by excessive nutrition at the chalazal end. A few 6-nucleate embryo sacs were seen with only one antipodal nucleus. In other cases more than three polar nuclei were seen with only one antipodal nucleus. In the light of Bambacioni's (1928) work, Schnarf (1931) reinterpreted Frisendahl's figures and concluded that the embryo sac is of the Fritillaria type. Maheshwari (1937) also states: "Frisendahl's (1912) figures show the 1+3 arrangement as well as a difference in the relative size of the micropylar and the chalazal pairs of nuclei of the 4-nucleate stage. There is, therefore, no doubt that the Fritillaria type occurs here, but this plant seems to be rather variable in its behaviour and, therefore, a re-investigation is likely to give interesting results".

At about the same time Zabban (1936) actually demonstrated the occurrence of the Fritillaria type of embryo sac in *Myricaria germanica*. Battaglia (1943) has confirmed it and showed that the occasional reduction in the number of nuclei, observed by Frisendahl, was due to the ommission of the last division at the chalazal end. Degenerations also occur sometimes affecting the chalazal nuclei of the embryo sac.

Mauritzon (1936) has investigated 6 species of *Tamarix*: *T. tetrandra*, *T. pentandra*, *T. gallica*, *T. aestivalis*, *T. africana* and *T. odessana*. *T. tetrandra* was more thoroughly worked out. He does not seem to have been aware of Bambacioni's (1928) work on *Fritillaria*. Maheshwari (1937) remarks "Since Mauritzon's figures do not give any indication of a 1+3 arrangement, nor a difference in size of the nuclei at the two ends of the embryo sac, it is not possible to go further into the question and a fuller investigation of the genus *Tamarix* seems to be desirable". Mauritzon was unable to interpret some of his own observations and stated that not infrequently he came across embryo sacs in which the number as well as the organization of the nuclei made it impossible to interpret them with certainty. He also found that the antipodals sometimes attained a considerable size after fertilization. In this connection, Maheshwari (1946a) suggests that the ephemeral or persistent nature of the antipodal cells may be due to two different modes of development. The triploid antipodals in embryo sacs of the Fritillaria type are likely to be better developed and more persistent than others. However, we did not find any such difference and the antipodals were in fact quite well developed irrespective of whether the embryo sac developed according to the Fritillaria, Drusa, or the Adoxa type. In post-fertilization stages of *T. ericoides* there was no trace of the antipodals, and in this respect our observations agree with those of Puri (1939) on *T. chinensis*.

Investigations of other species of *Tamarix* have yielded interesting results. The Fritillaria type of embryo sac has been reported in *T. ericoides* (Sharma, 1939, 1940) and *T. dioica* (Joshi & Kajale, 1936). In both these species some of the embryo sacs showed 1+2+1 arrangement (*T. ericoides*, Fig. 16; *T. dioica*, Fig. 7) but probably the nuclei become arranged subsequently in a 1+3 manner.

Pàroli (1939) and Puri (1939) have also reported the Fritillaria type of embryo sac in *T. gallica* and *T. chinensis* respectively. Some variations were often observed. Pàroli's (1939) Fig. 25 shows a 1+2+1 arrangement of megaspore nuclei. His Fig. 26 shows a single nucleus each at the micropylar and the chalazal ends and a large binucleolate nucleus in the centre of the embryo sac. Such a condition would lead to the Chrysanthemum cinerariaefolium type. In *T. chinensis*, Puri observed an embryo sac with 2 chalazal and 2 micropylar nuclei of similar size which by one more division could produce an 8-nucleate embryo sac of the Adoxa type. Other variations include 2+4, 2+5 or 2+6 arrangements, i.e. 2 nuclei at the micropylar and 4, 5 or 6 at the chalazal end. These may give rise to embryo sacs of the Drusa type. More than 2 polar nuclei were often present in some embryo sacs.

Battaglia (1941) reported four types of embryo sacs in *T. gallica* and three in *T. africana* in the following proportions:

	T. gallica	*T. africana*
Fritillaria type	90%	43%
Drusa type	3%	10%
Adoxa type	2%	Nil
Chrysanthemum cinerariaefolium type	5%	47%

We found all the four types in *T. pentandra* but only three in *T. troupii*:

	T. pentandra	*T. troupii*
Fritillaria type	38%	73%
Drusa type	8%	Nil
Adoxa type	48%	14%
Chrysanthemum cinerariaefolium type	6%	13%

Since the Fritillaria type occurs more commonly than others in *Myricaria* as well as in *Tamarix*, it may be considered to be characteristic of the family.

ENDOSPERM — According to Frisendahl (1912), in *Myricaria germanica*, the polar nuclei fuse only after syngamy. Endosperm development is very slow and only 8 nuclei could be counted in an embryo sac with even an advanced proembryo. The maximum number of endosperm nuclei is 16. Eventually the endosperm is completely consumed by the embryo.

Mauritzon (1936) reports Cellular (wall formation after the first division) as well as Nuclear endosperm in the same species and even in the ovules of the same flower of *Tamarix tetrandra*. He further states that even in those species where the endosperm is Nuclear, wall formation does take place in the end although the endosperm is only poorly developed. In one abnormal embryo sac of *T. tetrandra*, which showed a fairly large embryo, the endosperm was expected to be cellular but it showed such a condition only in the upper constricted part of the embryo sac between the embryo and the nucellar epidermis. The lower part contained about 10 free nuclei. Mauritzon interprets such a condition as transitional between the cellular endosperm of *Tamarix* and the feebly developed and quickly degenerating Nuclear endosperm of *Myricaria*[6].

Puri (1939) states that in *T. chinensis* the endosperm remains nuclear and the cellular condition is never attained. If so, probably this species will more aptly represent the transitional type between *Tamarix* and *Myricaria* but it would not be surprising if older ovules of *T. chinensis* also show cellular condition.

According to Sharma (1939) the endosperm of *T. ericoides* is poorly developed and only 10-15 endosperm nuclei are formed. He states: " the cellular endosperm that Mauritzon (1936) reports in *T. tetrandra* does not form here ". We have observed as many as 40 free endosperm nuclei in *T. ericoides* and although the endosperm starts as nuclear, wall formation does take place after the differentiation of the cotyledons. The endosperm cells are poorly cytoplasmic, do not contain any food reserves and are consumed by the embryo during maturation of the seed.

It may be concluded that in both *Tamarix* and *Myricaria* the endosperm is Nuclear and that it becomes cellular in *Tamarix* in later stages. Mauritzon's report of a Cellular endosperm in *T. tetrandra* appears to be erroneous.

SYSTEMATIC POSITION — Bentham and Hooker (1862) placed the family Tamaricaceae under the Subdivision Polypetalae, Series Thalamiflorae, Cohort Caryophyllinae, along with the families Caryophyllaceae and Portulacaceae, just before the Malvaceae. Hutchinson (1926) has raised it to the level of a separate Order Tamaricales — just before Passiflorales, Malvales and Cactales. Engler and Diels (1936) assign it to the Parietales and Wettstein (1935), Lawrence (1951) and

6. Mauritzon (1936) states: " *Tamarix tetrandra* zeigt eine interessante Variation in der Entwicklung des Endosperms, indem ich gefunden habe, dass es sowohl nach dem zellularen wie nach dem nuklearen Typus gebildet werden kann.". . . " Das nukleare Endosperm der gleichen Art (*T. tetrandra*) wird, wie die Fig. 5F-G zeigen, normal entwickelt. Da ich in allen älteren Samenanlagen zellulares Endosperm gefunden habe — obgleich häufig sehr schwach ausgebildet — muss man also annehmen, dass das nukleare Endosperm bei *Tamarix* im Gegenstaz zu *Myricaria* nach einiger Zeit zellular wird."

Rendle (1952) are in agreement with this.

Embryological studies have shown that the Fritillaria type of embryo sac does not occur in any family of the Order Parietales except the Tamaricaceae; nor is it found in any of the allied Orders. This suggests the need of a re-examination of the systematic position of this family. Further comparative work on representative genera included in different families of the Parietales as well as on the two incompletely known genera of Tamaricaceae, *Reaumuria* and *Hololachna*, is necessary before any satisfactory conclusion can be arrived at. For the present Hutchinson's erection of a separate Order Tamaricales seems to be justified.

Summary

The flowers of *Tamarix pentandra* and *T. troupii* are pentamerous and the superior, unilocular ovary contains a large number of bitegmic anatropous ovules.

The anther wall comprises the epidermis, fibrous endothecium, two middle layers and glandular tapetum. Reduction divisions are simultaneous and cytokinesis occurs by furrowing. The mature pollen grains are 2-celled.

The nucellar archesporium is 1- to 2-celled and a wall cell is cut off. The embryo sac is tetrasporic and in *T. pentandra* it may conform to the Fritillaria, Drusa, Adoxa, or Chrysanthemum cinerariaefolium types. In *T. troupii* the Drusa type has not been observed but the other three types are present. The frequency of the different types of embryo sacs in both the species has been worked out. Some abnormal embryo sacs have also been recorded in *T. pentandra*.

The endosperm is Nuclear and becomes cellular only in late stages. It is consumed by the developing embryo and none of it is left in the seed. The embryo has a massive suspensor. Polyembryony occurs sometimes, the additional embryo arising from a fertilized synergid.

After the appearance of the cotyledons the inner integument collapses and only the outer takes part in the formation of the seed coat. The nucellar epidermis persists as perisperm. The embryo occupies the entire space in the seed.

After fertilization the chalazal end of the ovule elongates and the epidermal cells develop into uniseriate hairs which form the plume on the surface of the seed.

It is a great pleasure to express our gratitude to Prof. P. Maheshwari for revising the manuscript, and to Prof. R. Souèges (University of Paris) for suggestions regarding development of the embryo.

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ON A RARE AND LITTLE KNOWN ALGA (*TYDEMANIA EXPEDITIONIS* WEBER V. BOSSE) NEW TO NANCOWRY

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The genus *Tydemania* with the type species, *T. expeditionis* was established by Weber van Bosse on specimens collected by her from the East Indian Archipelago during the 'Siboga' Expedition (Weber van Bosse, 1901, pp. 139-140). In 1905, Gardiner collected during the 'Sealark' expedition to the Seychelles an alga from the Chagos Archipelago and the adjacent islands in the Indian Ocean, which was first referred to *T. expeditionis* by Gepp (Gepp & Gepp, 1908, p. 174), but later on described as a new species, *T. gardineri* (Gepp & Gepp, 1911, p. 67). Recently Taylor (1950, p. 73) recorded *T. expeditionis* from Marshall Islands in the Pacific. There has been no report of this genus so far from any other locality.

During a marine algal survey of the Andaman and Nicobar Islands in the Bay of Bengal, the author collected from Nancowry (7·58 N. lat., 93·40 E. long.) in the Nicobar Islands, some very good specimens of *Tydemania expeditionis*. Very little is known of this rare and interesting species. The earlier accounts of the alga given by Weber van Bosse (1901, pp. 139-140; 1913, pp. 116), Gepp & Gepp (1911, pp. 65-68), Printz (1927, pp. 129) and Taylor (1950, p. 73) are rather brief and mainly of a taxonomical nature only. An examination of the specimens from Nancowry revealed, however, certain interest-

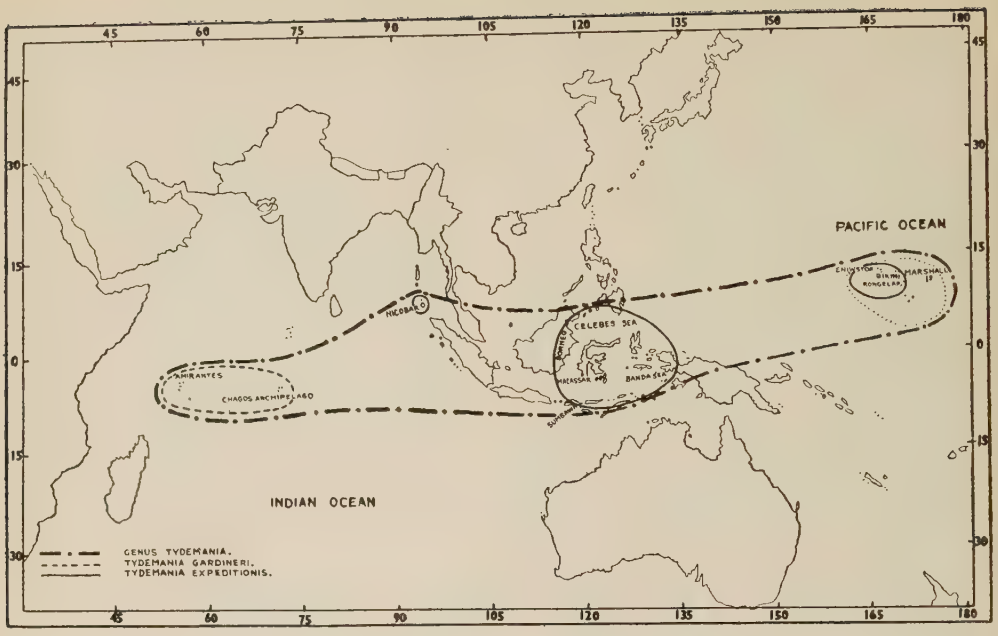


FIG. 1 — Map showing distribution of *Tydemania*

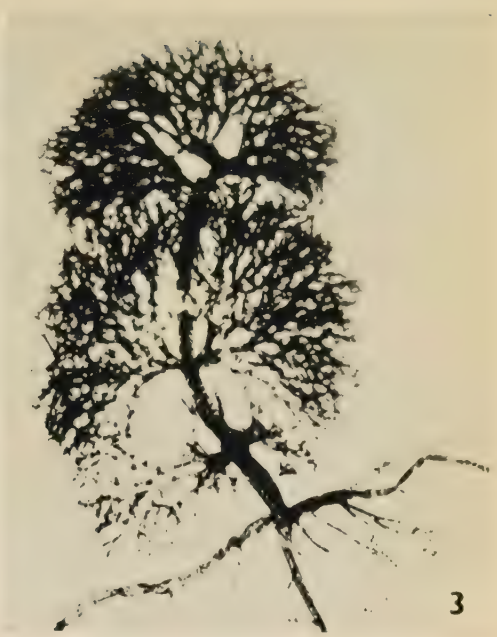
ing features which have not been recorded previously. The Nancowry alga is, therefore, described in some detail here below.

The alga was found growing attached to a coralline rock, in a somewhat sheltered bay. Several plants were growing forming a sort of a loose clump (Figs. 2, 10). The plants grew at a depth of two to three feet below low-tide mark and the shoots of the plants were constantly moving to and fro in the water due to wave action.

The alga is a large coenocyte, measuring up to 11 cm. in height and is somewhat feebly calcified. Two regions can be recognized in its thallus, (1) a '*prostrate system*' consisting of a thick-branched rhizome, creeping over the substratum and (2) an '*erect system*' consisting of a number of erect shoots growing from the upper side of the prostrate, rhizomous filaments. These upright shoots bear a series of contiguously placed spheroid to sub-spheroid, branched structures which give a characteristic appearance to the alga (Figs. 2, 10).

THE PROSTRATE SYSTEM—The prostrate system is made up of a creeping rhizome of long decumbent branched filaments. These are monosiphonous, thick-walled and tough and are constricted at shorter or longer intervals throughout (Figs. 12, 19, 20). They are 280-532 μ or more in diameter. They are attached to the substratum by a large number of rhizoids which are developed from the lower side of the filaments. The rhizoids are branched dichotomously or occasionally trichotomously and are either cylindrical or irregular in shape. They vary in thickness from 28-140 μ. In the majority of cases, the rhizoids are constricted at very short intervals especially at their base, and have a torulose or moniliform appearance (Figs. 12, 15, 17, 19). Frequently the free ends of some of the rhizoids branch in an irregular fashion to form a sort of a haptera (Fig. 12). The numerous rhizoids with the haptera formed by them constitute an efficient '*attaching system*'.

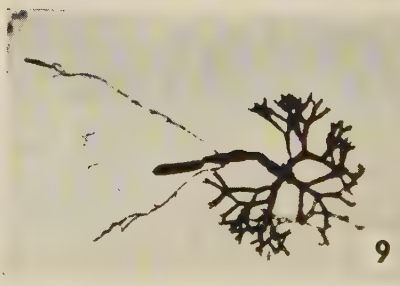
The growth in length of the basal rhizomous filaments is apical. The terminal segment of the rhizome, in its well-developed state, is generally long and



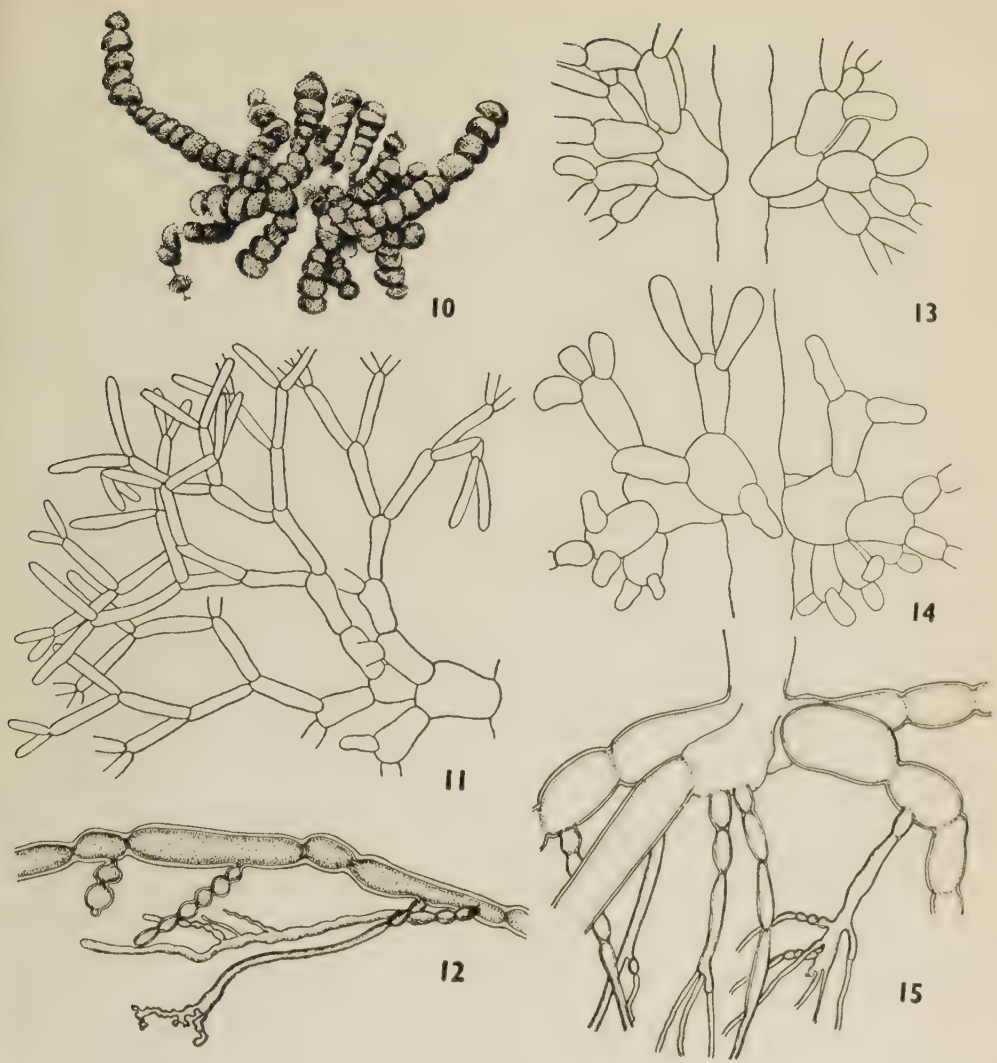
FIGS. 2-3 — Fig. 2. Photograph of a plant showing habit. × ¾. Fig. 3. Photograph of a young erect shoot with three glomeruli showing the cruciate appearance of rhizome portion at its base. Some of the branches of the glomeruli removed to show details. × 4½.

cylindrical with a more or less broadly rounded or obtuse end (Fig. 19). Sooner or later, this segment produces, at its free end, a small lobe which now becomes the terminal segment (Fig. 20). The new terminal segment thus formed soon grows further in length, and ultimately forms in its turn a fresh terminal segment. This process is repeated again and again and the growth of the creeping rhizome is thus regularly maintained. The rhizome is branched, and the branches may arise laterally from the sides of any of the segments behind the terminal one, and grow in a horizontal direction creeping on the substratum. In certain cases as will be shown further on, lateral branches are formed from the terminal segment also.

THE ERECT SYSTEM — The erect shoots are always formed by the terminal segments of the prostrate filaments. Just before the formation of the upright shoot, the growth of the terminal segment of the rhizome becomes temporarily arrested and the segment becomes richer in contents and thicker. It then forms on its dorsal side, a short thick lobe which becomes the primordium of the upright shoot. This primordial lobe soon grows out into a short thick erect axis perpendicular to the creeping rhizome (Figs. 4, 16 *d*). The apical portion of this short axis then becomes somewhat thicker than the portion below. At this stage, the apical portion of the axis stops its upward growth and gives rise to four lateral branches (primary lateral branches) (Fig. 16 *e*) which are situated more or less equidistantly round the axis just below its apical portion. Very frequently the number of these primary branches may be only three (Figs. 16, 17) or very occasionally even five. Each of these primary branches then divides trichotomously or dichtomously once or twice and forms branches of the second or third orders respectively (Figs. 13, 14, 16, 17). Very occasionally the primary branch does not divide at all, but grows and forms



FIGS. 4-9 — Fig. 4. Photomicrograph of a very young plant showing a very early stage in the development of a glomeruliferous shoot. × 4. Fig. 5. Photomicrograph of a very young plant showing a slightly advanced stage. The terminal portion of the axial filament of the erect shoot after forming one verticil of branches has grown out upwards again. × 3⅓. Fig. 6. A young glomeruliferous shoot showing one of the primary lateral branches grown out into a stoloniferous filament similar to the rhizome. ×3½. Figs. 7, 8. Photomicrograph of flabella. × 14. Fig. 9. Top view of a young glomeruliferous shoot showing one of the four primary lateral branches grown out into a stoloniferous filament with rhizoids, similar to the filaments of the rhizome. × 4.



FIGS. 10-15 — Fig. 10. A cluster of plants showing habit. × $\frac{1}{3}$. Fig. 11. Portion of a verticil of the erect shoot, showing a primary branch and the branches of higher orders. Note the first trichotomous and the later dichotomous branchings. × $16\frac{2}{3}$. Fig. 12. Portion of a rhizome with rhizoids. Note the moniliform base of the rhizoids and the haptera at the free end. × $16\frac{2}{3}$. Figs. 13, 14. Portions of young erect shoots, showing the axial filament and the primary branches and the branches of the second and higher orders formed by them. × $16\frac{2}{3}$. Fig. 15. Base of a glomeruliferous shoot showing the cruciate appearance of the prostrate filaments. × $16\frac{2}{3}$.

another segment which then divides either trichotomously or dichotomously (Figs. 17, 21). The subsequent branchings are, however, invariably dichotomous and in alternate planes (Figs. 11, 18). The primary lateral branches are generally broadly wedge-shaped and measure from 420-616 μ across (Figs. 16, 17 *e*). But the branches of the second, the third and the higher orders gradually become thinner and longer. The lower branches are up to 560 μ long, while the upper branches are 800-1400 μ long. The lower branches are about 165-270 μ in thickness, while

the ultimate branches are about 84 μ in thickness only, though occasionally they may reach a thickness of 112 μ. The branches formed by all the primary branches, ultimately result in a verticil of highly branched structures (Figs. 5, 17).

The axial filament, after forming the verticil of branches around its apex, resumes its growth and becomes elongated (Figs. 5, 17), and then forms another whorl of branched structures around its apex, in exactly the same manner as described above. This process is repeated several times, and a series of such whorls of branched structures are formed one above the other in regular succession (Fig. 3). The branches at each whorl get laxly inter-woven into a sort of a spheroid or sub-spheroid structure known as glomerulus (Figs. 3, 18). In a well-developed plant the axial filament may bear up to 16 or more such glomeruli at intervals of 4-6 or 10 mm. (Figs. 2, 10). Each glomerulus measures about 1 cm. high and 1-1·5 cm. broad. The central axis bearing the glomeruli measures from 560-784 μ in thickness. The fully developed glomeruliferous axial shoot is mostly unbranched (Fig. 2), but occasionally the axial shoot may be branched once or twice (Fig. 10).

The terminal segment of the rhizome, while forming the primordium of the erect shoot on its dorsal side, produces more or less at the same time two lateral lobes, one on either side of it and at right angles to it (Fig. 16, *b*, *b*1) and an anterior lobe (Fig. 16, *c*). The anterior lobe becomes the apical segment and continues the growth of the rhizome, while the two lateral lobes grow out horizontally into long creeping filaments at right angles to the main rhizome. As a result, the glomeruliferous shoot appears to be situated as it were on a cruciate base, made up partly by the portion of the main rhizome forming it, and partly by the two lateral horizontal branches formed by it (Figs. 3, 4, 6, 15, 16, 18). Very occasionally, only one lateral branch is formed by the segment which gives rise to the erect shoot and then this cruciate appearance of the base is not seen (Figs. 5, 17).

Flabella — A very careful examination of the prostrate filaments of numerous plants revealed the presence of a few flabella borne on the filaments (Figs. 7, 8, 22, 23). The flabella are very minute structures about 4-4·5 mm. high and resemble small plants of *Udotea javensis*. They are extremely rare in the Nancowry specimens, and are often covered by the tangle of rhizomous filaments and rhizoids, and foreign material and so are likely to be easily overlooked.

The flabella arise directly from the creeping rhizome at irregular intervals, either singly or in pairs (Figs. 8, 22). The flabella have a somewhat short thick moniliform stalk (stipite). They are monostromatic and composed of laterally connate dichotomously branched filaments The stalk of the flabella are 200-410 μ in thickness. The branches near the upper

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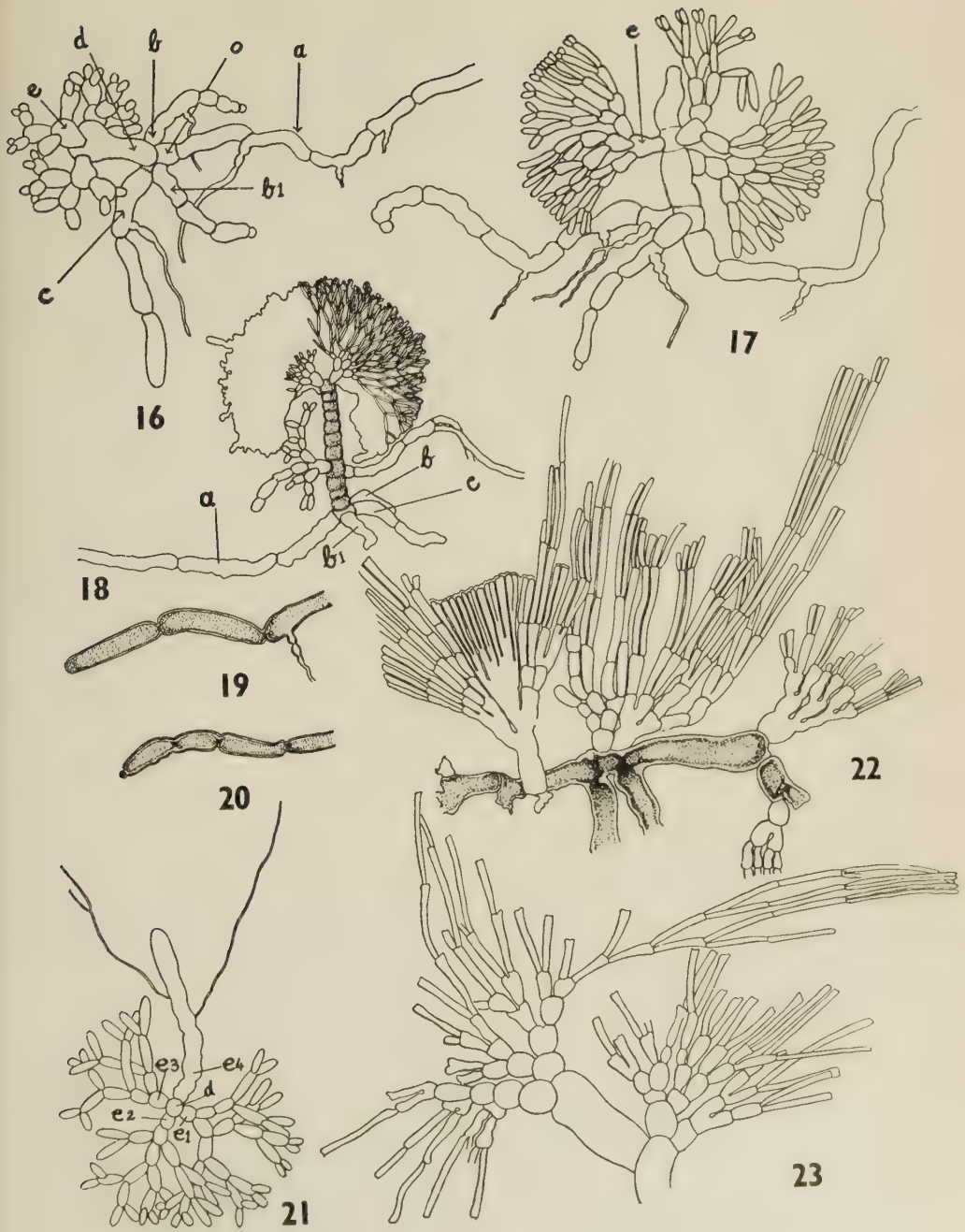
Figs. 16-23 — Fig. 16. A very early stage in the development of a young glomeruliferous shoot (*a*, main filament of the rhizome; *o*, original terminal segment of the main filament from which the erect shoot was formed; *b*, *b*1, lateral segments formed by the original terminal segment *o*; *c*, anterior segment formed by the original terminal segment; *d*, dorsal segment; *e*, primary lateral branch of the upright shoot). × 6. Fig. 17. A slightly advanced stage in the development of a glomeruliferous shoot showing the axial filament growing upwards again after forming one verticil of branches. × 7. Fig. 18. A more advanced stage in the development of a glomeruliferous shoot. At base is seen the cruciately shaped prostrate filament. In the verticil one of the primary-lateral branches has grown out into a stoloniferous filament. In the node higher up is a normal verticil. Some of the lateral branches of the two verticils are removed to show details. × 3. Fig. 19. Portion of rhizome showing the elongated terminal segment with its rounded tip. × 7. Fig. 20. Portion of a rhizome with a very small new terminal segment being formed at the tip of the older segment. × 7. Fig. 21. Apical view of a verticil of an erect shoot showing the axial filament *d* with four primary lateral branches, *e*1, *e*2, *e*3 and *e*4. One of the primary lateral branches *e*4 has modified into a stoloniferous filament with rhizoids. × 6. Figs. 22, 23. Flabella showing details of structure. Fig. 22, × 12. Fig. 23, × 14.



FIGS. 16-23.

end are narrow, elongated and delicate and are about 54·6 μ in thickness. In all the cases examined by me, the portions of the flabella more towards the base were more or less intact, but the more delicate upper portions were generally found lost or somewhat damaged.

In its general habit, the Nancowry alga agrees very well with the type-species *Tydemania expeditionis* from the Malayan Archipelago. But in the size of the prostrate filaments and of the basal and apical branches of the glomeruli, it differs from the type species in being generally larger throughout. In the type species, the prostrate filaments are 400-450 μ thick, whereas in the present alga they are 280-532 μ or even more in thickness. The primary branch of the upright shoot (i.e. the basal branchlet of the axial filament) in the type species, is only 240 μ thick, whereas in the present alga it is 420-616 μ in thickness. Similarly, the ultimate branches of the glomerulus in the type species are only 63 μ in thickness, whereas in the present alga they are usually 84 μ in thickness and occasionally up to 112 μ in thickness. The successive branchings of the glomeruli in the type species have been described as being only dichotomous. In the present alga, the first one or two divisions of the primary lateral branch are either trichotomous or dichotomous. Trichotomous branching has not been recorded in the type-species. The flabella in the type-species are, however, larger in size. They are 10-15 mm. long in the type-species, whereas in the present alga, they are very small and are only 4-4·5 mm. long. The upper cylindrical filaments of the flabella in the type-species are not less than 63 μ in thickness, whereas in the present alga they are only about 54·6 μ in thickness. The cruciate appearance of the prostrate filaments at the base of the glomeruliferous shoots are not reported in the type species.

Tydemania expeditionis in having a stoloniferous prostrate portion, with erect shoots growing from its upper side shows a certain amount of general resemblance to a *Caulepra* such as *C. verticillata*. This has a creeping rhizome which grows by apical growth and is attached to the substratum by means of numerous branched rhizoids formed on its lower side. From its upper side arise a number of erect branches (assimilators) which form a series of whorled branches. But the resemblance is only superficial and does not go any further. The two algae are not related at all.

Finally a short reference may be made to the geographical distribution of this rare and interesting genus. The genus *Tydemania* would appear to be confined to a rather narrow belt in the Tropical waters, its range being, North to South approximately between 10°N. and 9°S. lat. (19°lat.) and East to West, between 53°E. and 171°E. long. (118° long). The only two species belonging to this genus, have originated from two different geographical areas, which are rather widely separated. The principal centres of distribution of *T. expeditionis* and *T. gardineri*, as hitherto known, are the Malayan Archipelago and the Marshall Islands on the East and the Chagos Archipelago and Amirante Islands on the West of the Indian Ocean respectively (Fig. 1). The present discovery of *T. expeditionis* at Nancowry Island in the Bay of Bengal in between the geographical confines of the two species, is very interesting.

Summary

1. An account is given of *Tydemania expeditionis* Web. van. Bosse collected from Nancowry Islands in the Nicobar Islands in the Bay of Bengal. This is the first record of this alga from Indian territorial waters.

2. The alga consists of a prostrate system of branched creeping rhizome attached to the substratum by means of numerous rhizoids formed on its lower side, and an erect system consisting of erect glomeruliferous shoots and flabella arising from the upper side of the rhizome.

3. The geographical distribution of the genus and the structure and development of the glomeruliferous shoots and the flabella are described in detail.

I am greatly indebted to Prof. M. O. P. Iyengar, for his very valuable help, suggestions and kind criticisms of the paper.

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REVIEWS

ARBER, A. 1954. "The Mind and the Eye." Pp. 146. Cambridge University Press, London. 16*s*.

In ancient India the period of human activity was divided into four stages or *ashrams*. The last of these was the *sanyas ashram*, when one used to retire completely from his petty worldly pursuits and focus all attention on bigger and more absorbing problems. This was renunciation, believed to be the way to salvation. Dr. Arber appears to have become a *sanyasi* in so far as her mental pursuits are concerned. Standing on top of her lofty experience of over fifty years she looks back and analyses the biologist's approach to his own work and to philosophy. The analysis is so exhaustive and accurate that any researcher will find a lot therein which will apply to him in some form or another.

Dr. Arber recognizes six stages into which a researcher's work may be analysed. They are: (1) selection of a problem, (2) acquisition of factual data, (3) interpretation of the data and formulation of a hypothesis, (4) testing of the hypothesis, (5) writing of the results and (6) final contemplation. The first five of these form the subject-matter of five chapters constituting the first part of the book, while 'contemplation' is dealt with in the second part which also comprises five chapters. Each part is preceded by a brief 'Introduction'. At the end of the book there are listed about 350 valuable references which bear page numbers of the text where they are referred to as footnotes. These cover all branches of study — art, literature, philosophy, science and mathematics — and the text is studded with beautiful quotations from many a master mind.

At the outset the author emphasizes the importance of exercising great care in the selection of a research problem. "The mind languishes when faced with an enigma which lacks content, but, confronted by a problem worthy of its steel, it often displays powers which were previously quite unsuspected." Not only, therefore, should the problem have 'content' but also it should suit the temperament and talent of the worker, in order that it may bear fruit.

The second chapter, though brief, is most fascinating. It deals with the mode of discovery or the origin of hypothesis. After giving a vivid picture of Henri Poincare's famous hypothesis, Mrs. Arber discards it as impracticable from the biologist's stand point. According to her view, "new hypotheses come into the mind most freely when discursive reasoning — has been raised by intense effort to a level at which it finds itself united indissolubly with feeling and emotion. When reason and intuition attain to this collaboration, the unity into which they merge appears to possess a creative power which was denied to either singly." This is indeed a fine exposition of the discoverer's mind though the reviewer's own experience, which no doubt is very insignificant, seems to fit better within the framework of Poincare's picture. More often than not he had apparently forgotten all about the problem that defied every attempt at its solution when all of a sudden, at an auspicious moment as it were, he realized its explanation. The suggestion that there is some difference in the *modus operandi* of the mind of a morphologist and that of a mathematician does not appeal to the reviewer and he feels that both these explanations hold good under different situations.

The next two chapters are devoted to a detailed discussion of the role of logic and analogies in the testing of the proposed hypothesis. It is concluded that biological hypotheses, on account of their very nature, cannot be proved or disproved with certainty.

Regarding the communicating of the results to the world at large, the author draws pointed attention to certain handicaps which a researcher experiences. The

most stumbling of these, according to her, is the imperfection of the language. A written account at its best can be just a " linear sequence " whereas the thought, which it seeks to describe is in the form of a ' reticulum '. So the former can never do justice to the latter. Secondly, the mode of discovery in almost all branches of study is profoundly different from that of exposition. In many cases the discoveries are made intuitively, and " it is only afterwards that a public highway to them is constructed ". This highway has to be smooth and straight and, therefore, very different from what the researcher himself may have used while reaching the goal.

The author considers biological writing as an organic whole which can be seen emerging, " only when the factual material is fused in the crucible of thought ".

The second part of the book is devoted entirely to the last stage — the final contemplation in which all the previous phases " find their end and their justification ". Here the biologist goes beyond his individual observations and conclusions and assesses his general mode of thought and the relations of his work in a broader context. For achieving this objective he has to probe deeper into the nature of the various concepts, which he has so far taken for granted.

One such concept is ' truth '. He tries to determine now the exact implications of this word for himself. He realizes that in the earlier stages of his work he was primarily concerned with what is described as ' contingent ' or ' provisional ' truth — the main object of the physical scientist. But now he aspires to get a glimpse of Hegel's ' Wahrheit ' — " that truth in which the thought content is in agreement with its own essential character ". He recognizes that ' error ' is just an imperfect form of truth and that there is " no duality of truth and falsity to reconcile ".

Another concept that attracts his attention is that of the thesis and antithesis. He analyses a number of these pairs, which he is likely to encounter in his search for truth, and concludes that they are just complimentary to one another — their apparent antagonism being due to their imperfection. " The antithetic pairs which necessarily show as dual in the world of phenomena, to which we have access through the senses, are each fused into a unity in the world of thought, to which the mind has the key." The biologist thus would not allow his ideas to be " distorted by an exclusive adherence to one or the other of the two partial conceptions ", the thesis and the antithesis.

Having thus realized the multi-faceted character of truth the biologist finds it all the more difficult to express his ideas in a simple ' linear sequence '. On the other hand, he now observes that the " to-and-fro quality " of the dialogue or written correspondence can do better justice, than simple speech, to the complex synthesis of of antitheses.

Next our ' biologist ' undertakes to unearth his basic assumptions. He realizes that his very approach to nature is based on the assumption that it is " intelligible to reason ". There are many other basic assumptions that come to his mind. He does look at them with a critical and unprejudiced eye, but within the short span of his life he is unable to verify them all for himself. Nevertheless he becomes conscious of the fact that some of his most cherished explanations and theories are still based on assumptions. And this is enough to make him feel humbler than he was ever before.

In the end attention is focussed upon " the two roads to reality open to the biologist — the way of the intellect and the way of the eye ". As to the relation between these two approaches the biologist is in complete accord with Kant who is quoted to have written: " The understanding can intuit nothing, the senses can think nothing. Only through their union can knowledge arise."

Few of us are fully aware of the stages of our mental life while we are passing through them. A prolific writer and a stimulating thinker, Mrs. Arber, has rendered a unique service to all searches after truth by giving this valuable analysis which has the details and accuracy of an autobiography. And what is more, this analysis is followed by an equally valuable synthesis of metaphysical and scientific thinking. She has done a fine job of interpreting the scientist to the philosopher

and the philosopher to the scientist. And this is the ideal she places before all students of biology, which branch she considers as part and parcel of 'Natural Philosophy' rather than of 'Natural Science'. By her mature thought and long contemplation she has indeed illuminated, more than anybody else, the path of the biologist 'to reality'.

We express our gratitude to Dr. Agnes Arber and congratulate the publishers for bringing out this handy, valuable guide to every searcher after truth.

V. PURI

FOGG, G. E. 1953. "The Metabolism of Algae." Pp. 149. Methuen & Co. Ltd., London. 8*s*. 6*d*.

ALTHOUGH a good deal of physiological work has been done on algae, especially on photosynthesis and permeability, there has not yet appeared any general summary of the distinctive features of algal physiology except the chapter by Blinks in Smith's "Manual of Phycology", and a brief article by Myers in the "Annual Review of Microbiology". Dr. Fogg is an active worker in this branch and has had the good fortune of having taken his lessons from Professors F. F. Fritsch and W. H. Pearsall, both masters in the line although with different interests. He has tried to integrate information which will be of considerable interest to a student of botany, microbiology and biochemistry.

As the reviewer understands, the Methuen monographs are meant for the general reader with a biological background. Unfortunately Dr. Fogg's treatise is not written so simply. It can be read with advantage only by one who comes close to being a specialist. It offers a contrast to some of Professor Pearsall's own writings which are quite learned, and yet not too obscure for the general reader.

P. MAHESHWARI

CRANWELL, L. M. 1952. "New Zealand Pollen Studies, the Monocotyledons, a Comparative Account." Bull. Auckland Inst. & Mus. **3**: 1-91. Harvard Univ. Press. 32*s*. 6*d*.

THE new science of palynology is making rapid strides. The fact that many spore characters may be retained undamaged not only in herbarium specimens but also in fossils gives special importance to such organs in palaeo-systematic studies.

Miss Cranwell's work deals with the pollen grains of a large number of cotyledons of the New Zealand flora. The task is difficult for the range of diagnostic characters in this group is rather small, and sometimes it is difficult to recognize even the family to which a sample belongs. At the same time, "just as one can recognise the familiar face in the crowd, so can one pick out the grains of certain genera in some atmospheric or even fossil preparation, for instance".

The monograph is illustrated with eight magnificent plates, detailed descriptions, a seven-page bibliography and an exhaustive index. There is no 'Summary', but a useful tabular survey of salient pollen characters is provided at the end. The work is thoroughly commendable in its scope as well as its thoroughness.

P. MAHESHWARI

BRIMBLE, L. J. F. 1953. "Intermediate Botany." 4th Ed. Macmillan & Co. Ltd., London. 20*s*.

MR. BRIMBLE'S popular text, revised and rewritten in collaboration with Drs. S. Williams and G. Bond of the University of Glasgow, has now appeared in a greatly enlarged form and in a new cover. It is written for candidates appearing for the General Certificate of Education and for pre-University examinations such as Intermediate Science, Agriculture, Horticulture and Medicine and has to be judged in that light.

The first chapter deals with the difference between living and non-living things; and between plants and animals. The second, a general survey of the plant kingdom, is enlivened by a photograph of the roadway cut through a Californian *Sequoia* and another of the Big Tree of Tule (*Taxodium mucronatum*). While the line drawings are effectively reproduced, this is unfortunately not always true of the half-tones. Chapter 3 gives a useful historical account of the development of botany right from the days of Theophrastus. The 4th describes the parts of a

flowering plant and the 5th the modifications of the root, stem and leaf. In chapters 6th, 7th, 8th and 9th the author takes a plunge into some aspects of plant physiology. His treatment of these is extremely lucid and everywhere the applied and historical aspect of things has been kept in the forefront. Chapter 10 deals with the microscopic structure of cells and tissues after which we again have a physiological chapter on absorption of water and dissolved substances. Chapters 12, 13 and 14 give a straightforward description of the functional anatomy of the root, stem and leaf. One wishes here that the three tiny photographs on page 135 had been omitted; they do not clearly show what they are intended to do and line drawings would have been much better. In chapter 15 we come to photosynthesis. Here the author not only gives the usual stuff but also discusses modern views on the mechanism of carbon assimilation and summarizes the work of Ruben and of Benson and Calvin using radioactive isotopes and chromatographic methods. Chapter 16 covers transpiration and internal movement of water and solutes and chapter 17 deals with respiration and fermentation. The author mentions the respiratory quotient as a useful indicator of the nature of the substrate but does not discuss the variations in its value when fats or organic acids are consumed. In discussing the mechanism of respiration there is no mention of the work of Krebs. Possibly this has been omitted because of the fear that elementary students may not be able to understand or appreciate it. A fairly detailed account of carnivorous plants is presented in chapter 18, followed by two chapters on growth and movements. Only a bare mention is made of photoperiodism and vernalization; nor has more than a small paragraph been devoted to the many applications of hormones in agriculture and horticulture. In the chapter on sexual reproduction in flowering plants, the figures used to illustrate the development of the male gametophyte are old-fashioned and unsatisfactory; no cytoplasmic sheath is shown around the generative nucleus and the structures labelled as male cells appear as nuclei only. A similar criticism may be made of Fig. 209 where the first two drawings ought to have shown traces of the three degenerating megaspores and there should have been a large central vacuole at the 2-and 8-nucleate stages of the embryo sac. On page 269 it is mentioned that the central fusion nucleus undergoes free nuclear divisions to give rise to endosperm nuclei. This is true of a large number of angiosperms but even in an elementary book it is perhaps appropriate to state that in most members of the Sympetalae and in many other angiosperms the endosperm is cellular right from the start.

Chapter 23 gives an excellent account of the life histories of some important genera of the algae at the end of which half a page is devoted to their economic uses. Throughout, the author's outlook is so fresh and modern that here one is surprised to find no mention of *Chlorella* as a possible source of food. Chapter 24, dealing with bacteria, fungi and lichens, also includes the parasitic angiosperms like *Cuscuta, Lathraea, Orobanche, Rafflesia, Viscum* and some members of the Scrophulariaceae, and the saprophytic *Neottia* and *Monotropa*.

Chapters 25 and 26 are concerned with the bryophytes and pteridophytes, and 27 with gymnosperms. In the third drawing in Fig. 321 prothallial *cell* should read as *cells*. The concluding chapters are devoted to a brief account of evolution, genetics, ecology and classification.

The reviewer considers the book to be one of the few good texts on the subject. It clearly reveals the breadth of vision of the author and his ability to make the subject interesting by constantly stressing its inter-relationship with human beings and world economy. The price is quite reasonable and everywhere there are such exhaustive suggestions for practical work that no companion volume is required and the book is complete in itself.

P. MAHESHAWRI

HAUPT, A. W. 1953. "Plant Morphology." Pp. 464. McGraw-Hill Book Company Inc., New York. $8.00

BEFORE attempting the present work the author already has two other books to his credit: "An Introduction to Botany"

and " Laboratory Manual of Elementary Botany " — all published by the McGraw-Hill Book Company.

When a research botanist undertakes to write a book, it is implied that a good deal of original material will be presented. In the words of the author himself " more than two-thirds of the illustrations are original, and most of these have not hitherto been published elsewhere ". These are all well chosen and have been carefully executed. There are a number of photographs, all of a high order.

Morphology undoubtedly covers the largest field of plant science " and may almost be said to be its very soul ". To write a handy book on the morphology of the plant kingdom is no easy task and Professor Haupt has attained it with commendable success. Various groups of plants and representatives of each group follow an evolutionary sequence. For a proper understanding of the growing complexity in structure, reproduction and development, the plants have to be studied in order of their progressive evolution. Such a co-ordinated account is presented in this book for the first time. It will be found useful by the Indian graduate students up to the Honours standard. The style is engaging and the language is simple. I have no doubt that it will prove stimulating to the student as well as the teacher.

The book comprises 10 chapters. The first is an introductory account dealing with the classification of plants and plant life of the past. Chapters 2 and 3 are devoted to algae, 4 to fungi, 5 to bryophytes, 6 and 7 to pteridophytes, 8 and 9 to spermatophytes, and 10 to evolutionary tendencies including evolution of sex and alternation of generations. In the end there is a selected list of references and a glossary of technical terms.

Many readers will perhaps disagree with the system of classification followed here but classification is merely a matter of convenience. The author's objective has been to present essential features of different groups of plants which the student may further supplement by looking up some of the references at the end of the book.

In one important detail the book seems to be lacking. If each chapter were followed by some objective questions, the student would have a chance to check up his acquired knowledge. Another useful addition may be the inclusion of illustrations depicting the phylogenetic relationships of the various representatives of different plant groups.

B. M. Johri

CROCKER, W. & BARTON, L. V. 1953. " Physiology of Seeds. An Introduction to the Experimental Study of Seed and Germination Problems." Pp. 267. Chronica Botanica Company, Waltham, Mass. $6.50.

It is said that on a dry weight basis seeds constitute 80 per cent of the world's food supply. It is, therefore, good to have a broad and up-to-date summary of our present knowledge of seeds and their germination in a volume of moderate size. Ecological topics such as seed dispersal are omitted and only a brief account is given of the structure of the seed. The chief emphasis is placed on physiological studies, especially those carried out in the laboratories of the Boyce Thompson Institute of which the senior author (now dead) was director for many years.

There are 17 chapters each with an exhaustive bibliography, the total number of citations exceeding 1,100. The first part deals with the factors affecting germination, dormancy and the metabolic changes in germination. Next follow accounts of vernalization, embryo culture and seed transmission of disease. All of these are extremely satisfactory, but those on water relations, respiration and storage of seeds might perhaps have been slightly enlarged with advantage. There are 2 chapters devoted to a listing of the chemical compounds found in seeds.

The coverage of many of the topics discussed is unusually complete. In the part on factors affecting germination mention is also made of the effects of the moon, although the evidence is inconclusive and far from satisfactory. Concerning atomic rays, the authors quote the effects of the atomic bomb test of July 1946, on corn. Although the grains were not killed, the plants grown from

them were defective and abnormal resembling plants grown from X-rayed seeds. Regarding rapid viability tests, the authors do not consider the tetrazolium test to be dependable and recommend the embryo culture method. Of the causes usually held responsible for seed deterioration a degeneration of the nuclei of the cells of the embryo is considered to be the most important. On the practical significance of vernalization it is stated: "Vernalized winter wheat might give better production than non-vernalized winter wheat in certain cases, but it has never proved its superiority over spring varieties."

On the whole the book is most useful and fills a long felt requirement of English-speaking botanists.

P. MAHESHWARI

HESLOP-HARRISON, J. 1953. "New Concepts in Flowering-Plant Taxonomy." (The Scholarship Series in Biology.) Pp. 135. William Heinemann Ltd., London. 6*s*.

ALL botanists will be indebted to Dr. Heslop-Harrison for this brief but very interesting summary of modern viewpoints on the contacts between taxonomy, ecology and cytogenetics.

After an introductory chapter on the development of taxonomic concepts, the author devotes 15 pages to a consideration of the plasticity of the phenotype and concludes that for a given genotype there is a considerable range of environments (the tolerance range) in which it can successfully develop to maturity. The phenotype produced differs in its structure and physiology in accordance with the environment, but the range of phenotypic expression is itself genetically determined. In all cases it is the reproductive organs of plants which are least subject to modification and therefore have the greatest taxonomic value.

The bulk of the remaining space in the book is devoted to a discussion of the kinds of variability and the understanding of breeding systems and cytology in relation to taxonomy. Examples are quoted from a wide range of angiosperms. The last chapter summarizes the relationship between experimental and orthodox taxonomy. It is explained that the basic aim of experimental taxonomy is not the production of normal classifications. It is concerned instead with the identification of evolutionary units, the experimental determination of their genetical inter-relationships and the role of environment in their formation.

The book is moderately priced and the treatment is admirable.

P. MAHESHWARI

BROWN, R. & DANIELLI, J. F. (Editors). 1953. "Symposia of the Society for Experimental Biology. VII. Evolution." Pp. 448. Cambridge University Press. 45*s*.

THIS volume comprises certain papers read at a Symposium of the Society for Experimental Biology held at Oxford in 1952. It is the seventh of an annual series of Symposium reports. There are 23 articles on evolutionary topics. Since each author confines himself to a discussion of his own topic and there are neither any cross references nor an index the book is not one which can be read by the fireside or in the garden. It requires many days of careful study and quiet thinking. Further, the very richness of ideas and of approaches and viewpoints makes it impossible to summarize the contents in a brief review.

Of all the contributions the most interesting is the ten-page "Foreword" written by J. B. S. Haldane who gives a gist of all the papers and presents his own viewpoint here and there.

P. MAHESHWARI

RAMSBOTTOM, J. 1953. "Mushrooms and Toadstools. A study of the Activities of Fungi." Collins, London. 30*s*.

THIS is a wonderful book for the botanist, artist, mythologist, as well as connoisseur of table delicacies. It is also of interest to the timber dealer and the owner of a wooden house because of its very clear account of dry rot and how to overcome it. The last chapter gives an enchanting account of the discovery of penicillin.

In the reviewer's opinion this is one of the most attractive, popular (and yet scientifically accurate) books he has come across. The author has had a lifetime of experience of fungi, both in the laboratory and in the field, and is a fine writer. Every plant lover should have a copy on his shelf.

P. MAHESHWARI

WARDLAW, C. W. 1952. "Phylogeny and Morphogenesis. Contemporary aspects of botanical science." Pp. 536. Macmillan & Co. Ltd., London. 42*s*.

THE morphologist is often regarded as a relic of a 50-year old period — a person with less inventive genius and with an inadequate knowledge of plant physiology and biochemistry who still plods along in an overworked field which is of little importance in the present set-up of things. Is this really so? Professor Wardlaw's book provides the answer.

In the middle of the 19th century Wilhelm Hofmeister began a study of the embryology of the bryophytes and pteridophytes and extended his work even to the gymnosperms and angiosperms. He showed in 1851 that all these possess a common life cycle characterized by the same critical events and developmental phases and a recurrent alternation of generations. This work, published in 1862 under the title "Higher Cryptogamia" was a landmark in the development of botanical science.

Hofmeister's researches were not of a merely descriptive type. He always enquired: "How does the observed form come to be? What internal and external factors determine specific structural organization?" These queries became the starting-point of a fertile field of research, the so-called causal morphology which was so ably continued and elaborated by Sachs.

The publication of Darwin's "Origin of Species" followed by the enunciation of the theory of descent shifted the emphasis to other channels and Hofmeister's causal morphology was replaced by the "comparative morphology" of students of evolution. The anatomical and histological information gleaned from fossils provided material for comparison with living plants and began to be utilized for the construction of "genealogical trees" and phylogenetic systems. Bit by bit a fairly coherent, although largely speculative and in many ways incomplete account of the evolution of land plants, became available. Morphology and physiology began unfortunately to be pursued as independent and separate disciplines and the synthetic approach which was so characteristic of Hofmeister, Sachs, Nägeli and Hanstein lost so much ground that Goebel and Bower were perhaps its only representatives in the early part of this century.

Professor Wardlaw's book attempts to integrate the data of morphology, physiology and genetics, to present new concepts and hypotheses, and to relate these to those held formerly. The emphasis is throughout on the pteridophytes but other groups are not ignored.

The author concludes that morphology has passed through a period of transitory neglect and the position is fast changing. He says: "New ideas and techniques are opening new doors and broad vistas will certainly be the reward of those who maintain their effort in this widest of all fields of biological scholarship." We may hopefully envisage that morphology and physiology would advance side by side and that specialization in these branches would take place only against a mature background. In university departments it will be educative for physiologists to do some teaching in morphology and morphologists to take part in work on physiology. Only thus will develop a synthetic approach, productive of more radical advances in either field.

P. MAHESHWARI

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STOKEY, A.G. 1948. The gametophyte of *Actiniopteris australis*. J. Indian Bot. Soc. **27**: 40-49.

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